FEMS 2017

7TH CONGRESS OF EUROPEAN MICROBIOLOGISTS

JULY 9-13, 2017 VALENCIA, SPAIN

FEDERATION OF EUROPEAN MICROBIOLOGICAL SOCIETIES

FEMS

ABSTRACT BOOK

Scan this QR code to view the FEMS 2017 App

In Association with SEM | 26th Congress of the Spanish Society for Microbiology

FEMS7-3323
Emerging infectious diseases and how to cope with them

INNOVATIVE THERAPY FOR MYCOBACTERIAL DISEASE
S.T. Cole[1]
[1]*EPFL, Science de la Vie, Lausanne, Switzerland*

Innovative therapy for mycobacterial diseases

Stewart Cole
Global Health Institute, Ecole Polytechnique Fédérale de Lausanne
CH-1015 Lausanne, Switzerland

Tuberculosis (TB) was among the first infectious diseases to be rationally treated and the golden age of TB drug discovery led not only to new antibiotics but also to the development of combination therapy. No new TB drugs had been developed since the 1960s until recently but, in response to the HIV-pandemic and widespread resistance to first- and second-line drugs, a pipeline of candidate TB drugs has been established. Some of these drugs offer potential for the treatment of other mycobacterial diseases such as leprosy, Buruli ulcer and NTM infections. The ATPase inhibitor bedaquiline is especially promising followed by benzothiazinones, highly potent compounds that kill *Mycobacterium tuberculosis* by blocking a critical step in cell wall biosynthesis. The potential impact of these and other new candidate drugs will be discussed.

FEMS7-0496
Climate change

THE SUBZERO MICROBIOME: SEASONAL DYNAMICS OF BACTERIAL COMMUNITIES IN ARCTIC TUNDRA SOILS

M. Häggblom[1]
[1]*Rutgers - The State University of New Jersey, Biochemistry and Microbiology, New Brunswick, USA*

A substantial portion of the global soil carbon pool is stored in Arctic and boreal ecosystems and decomposition of this currently sequestered pool of soil organic carbon is expected to be a significant contributor to atmospheric chemistry and climate. A large portion of Arctic and boreal soil ecosystems are permanently frozen or only experience thaws for a brief time in the summer and due to the short warm season degradation of soil organic carbon in tundra soils has historically been slow and incomplete. Microbial life, however, continues into the subzero temperature range, and this activity contributes to carbon and nitrogen flux in and out of ecosystems, ultimately affecting global processes. The divergent life-styles of different microorganisms will be reflected in their ability to function in frozen soils and in their responses to environmental perturbations, leading to seasonal dynamics of activities. We seek to delineate the subzero active microbial community in Arctic tundra soils and discern their roles in soil organic matter degradation and to understand how different members of the microbiome modulate their responses to variations in temperatures and pulses of C and N substrates. Using a stable isotope probing (SIP) approach, we have shown that bacterial genome replication occurs at temperatures from 0 to -20 °C in seasonally frozen tundra soils, as well as in permafrost. Microbial degradation of plant litter is also substantial in frozen soils. Our findings indicate that Arctic and boreal soils impact greenhouse gas production/consumption while frozen, as well as when thawed. By understanding these processes in frozen soil systems, we hope to better predict the extent of C and N released as greenhouse gases which subsequently contribute to climate change.

FEMS7-2396
Climate change

MICROBIAL COMMUNITIES AND METHANE DYNAMICS IN ACTIVE LAYERS OF ARCTIC SOIL PERMAFROST ECOSYSTEMS

A.T. Tveit[1], T. Urich[2], M.M. Svenning[1]
[1]Arctic and Marine Biology, Microorganisms and Plants, Tromsø, Norway
[2]Institute of Microbiology, Microbiology, Greifswald, Germany

Permafrost soils store large amounts of organic carbon that is a source of the greenhouse gases (GHG) methane (CH_4) and carbon dioxide (CO_2). The active soil layer above permafrost consists of stored plant tissue mainly of mosses and/or vascular plants, primarily grasses and sedges contributing to polymers of different complexity. This active layer inhabit a diverse community of microorganisms with members from all three domains of life (Bacteria, Archaea and Eukarya), which cooperate for degradation of the stored organic matter to the greenhouse gases (GHG) methane (CH_4) and carbon dioxide (CO_2). The majority of active microbes belong to the phyla Actinobacteria, Bacteriodetes, Chloroflexi, Firmicutes, Proteobacteria, Verrucumicrobia, Planctomycetes, and Cercozoa. Microbes within the genera *Methanomicrobiales*, *Methanobacteriales* and *Methanosarcinales* perform CH_4 production. *Methylobacter* is further a key genus for the oxidation of CH_4. The carbon flow of this Arctic ecosystem, leading to GHG emissions, is dependent on how this microbial network community respond to climate changes.

The anaerobic microbial community and network metabolism were studied in response to a temperature gradient ranging from 1 to 30 °C by combining metatranscriptomic, metagenomic, and targeted metabolic profiling. The CH_4 production rate at 4 °C was 25% of that at 25 °C and increased rapidly with temperature, with associated changes in microbial communities, metabolic network of soil organic carbon decomposition, and trophic network. Arctic peat microbiota responds rapidly to increased temperatures by modulating metabolic and trophic interactions such that CH_4 is always highly produced.

FEMS7-0366
Climate change

REEF MICROBIAL SYMBIOSIS IN A CHANGING CLIMATE

N. Webster[1], H. Luter[1], E. Emmanuelle Botte[1], H. Bennett[1]
[1]Australian Institute of Marine Science, A healthy and resilient GBR, Townsville, Australia

Marine sponges contain complex and diverse microbial communities that contribute to the health of their hosts. Environmental conditions which disturb the distribution, abundance or function of sponge microbes can therefore have significant consequences for host fitness and survival. Climate change scenarios predict increases in sea surface temperatures (SST) and decreases in oceanic pH during the coming century. Using a combination of experimental research and data collected from natural CO_2 seeps, we have explored how elevated SST and ocean acidification (OA) impact sponge symbiosis. Sponges at CO_2 seeps have had a lifetime of exposure to high CO_2 therefore these sites are 'natural laboratories' for OA research, allowing us to answer questions of long-term acclimatization in the microbiome. Whilst the sensitivity of the sponge microbiome to climate change varies between species, a breakdown in symbiotic function which occurs in the early stages of climate stress for some species has been clearly linked to declining host health, and in some instances mortality. The application of genomic, transcriptomic and proteomic approaches to these model marine symbioses allows us to explore the functional implications of environmental stress for sponges and thereby better predict how they will acclimate and adapt to a changing climate.

FEMS7-3294
Biotransformation

ENZYME DISCOVERY, ENGINEERING AND APPLICATION IN THE SYNTHESIS OF CHIRAL COMPOUNDS AND CASCADE REACTIONS

U. Bornscheuer[1]
[1]*Institute of Biochemistry, Dept. of Biotechnology & Enzyme Catalysis, Greifswald, Germany*

This lecture will highlight principle strategies and current challenges in enzyme discovery and protein engineering [1]. For the synthesis of chiral amines, we performed an *in silico* analysis and identified a toolbox of novel (*R*)-selective ATAs [2] as well as (*S*)-selective enzymes from a structure-guided search [3]. More recently, we could engineer (*S*)-selective ATA for the acceptance of bulky ketones for the asymmetric synthesis of a set of important chiral amines [4, 5]. We also established cascade reactions and developed for instance the efficient synthesis of e-caprolactone-oligomers through combination of three enzymes [6].

[1] Bornscheuer, U.T., et al., *Nature*, **485**, 185-194 (2012); Kazlauskas, R.J., Bornscheuer, U.T., *Nat. Chem. Biol.*, **5**, 526-529 (2009); Lutz, S., Bornscheuer, U.T. (Eds.) *Protein Engineering Handbook*, Wiley-VCH, Weinheim (2009, 2012).

[2] Höhne, M. et al., *Nature Chem. Biol.*, **6**, 807-813 (2010); Schätzle, S. et al., *Adv. Synth. Catal*., **353**, 2439-2445 (2011).

[3] Steffen-Munsberg, F. et al., *ChemCatChem*, **5**, 150-153 (2013); *ChemCatChem*, **5**, 154-157 (2013)

[4] Nobili, A. et al., *ChemCatChem*, **7,** 757-760 (2015).

[5] Pavlidis, I., et al., *Nature Chem*., **8**, 1076-1082 (2016); Weiß, M.S. et al. *Org. Biol. Chem*., **14**, 10249-10254 (2016).

[6] Schmidt, S., et al., *Angew. Chem. Int. Ed.*, **54**, 2784-2787 (2015).

FEMS7-3319
Biotransformation

INTEGRATED SOFTWARE TOOLS AND LAB-ON-CHIP TECHNOLOGIES FOR BIG DATA IN BIOLOGY

J. Damborsky[1]
[1]*Masaryk University- Faculty of Science, Loschmidt Laboratories, Brno, Czech Republic*

Vanacek, P., Hon, J., Babkova, P., Buryska, T., Vasina, M., Chaloupkova, R., Bednar, D., Martinek, T., Prokop, Z., Damborsky, J., Integrated Software Tools and Lab-on-chip Technologies for Big Data in Biology

Millions of new protein sequences are being discovered at an incredible pace, representing an inexhaustible source of novel biocatalysts. The genetic databases are doubling every 15 months in postgenomic era [1]. The annotation of a large portion of this data lacks proper information on their biological function or includes no functional information at all. We have recently developed an integrated system for automated *in silico* exploration of protein functional diversity and robotic experimental characterization of attractive family members [2]. The workflow, which consists of: (i) identifying relevant genes by sequence and structural bioinformatics, (ii) expression analysis and activity screening, and (iii) complete biochemical and biophysical characterization, was validated against the haloalkane dehalogenase family. During the first round of the screening (carried out in 2013), the sequence-based searches identified 658 putative dehalogenases. Experimental characterization of 20 hits provided the most catalytically proficient native haloalkane dehalogenase enzyme to date, the most thermodynamically stable enzyme with melting temperature 71^{o}C, cold-adapted enzymes showing activity at near-to-zero temperatures and a new biocatalyst degrading sulfur mustard. The enzymes discovered originated from Bacteria and Eukaryota, and, for the first time, from Archaea. The second round of the screening (carried out in 2017), resulted in 520% increase of putative dehalogenases. In this round, the slowest steps in the experimental part of the platform will be replaced by newly developed lab-on-chip assays, requiring only tiny fractions (mL) of a protein material and providing dramatic increase in the throughput. Established strategy, software code and microfluidic chips can be adapted to other enzyme families, paving the way towards functional characterization of proteins produced based on data from next-generation sequencing technologies.

[1] Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Rapp, B.A., Wheeler, D.L., 2002: GenBank, Nucleic Acids Research 30: 17–20.

[2] Vanacek, P., Sebestova, E., Babkova, P., Bidmanova, S., Daniel, L., Dvorak, P., Stepankova, V., Chaloupkova, R., Brezovsky, J., Prokop, Z., Damborsky, J., 2017: Exploration of Enzyme Diversity by Integrating Bioinformatics with Laboratory Robotics (submitted).

FEMS7-0182
Biotransformation

DE NOVO ENZYME CASCADES IN CELL FREE AND WHOLE CELL SYSTEM BIOCATALYSIS

S. Flitsch[1]
[1], *United Kingdom*

The combination of sequential biocatalytic reactions in non-natural synthetic cascades is a rapidly developing field and leads to the generation of complex valuable chemicals from simple precursors. As the toolbox of available biocatalysts continues to expand, so do the options for biocatalytic retrosynthesis of a target molecule, leading to new routes employing enzymatic transformations. The implementation of such cascade reactions requires careful consideration, particularly with respect to whether the pathway is constructed *in vitro* or *in vivo*. This lecture will showcase three successful implementations of *de novo cascades* and discuss the relative merits of *in vitro*, *in vivo* or hybrid approaches to building biocatalytic cascades. We also highlight the factors that influence the design and implementation of purely enzymatic or chemo-enzymatic, one-pot, multi-step pathways.

Refs: **ACIE** 2016, 55, 1511; **Nature Chemistry** 2014, 6, 65; **JACS** 2012, 134, 4521, **ACS Catalysis** 2017, 7, 710-724.

FEMS7-0170
Biotransformation

ENZYME ENGINEERING STIMULATED BY CHEMICAL AND BIOLOGICAL DIVERSITY

B. Hauer[1]
[1], *Germany*

Enzyme Engineering stimulated by biological and chemical diversity.

Bernhard Hauer, University of Stuttgart

Industrial Biotechnology holds the promise to manufacture chemical compounds based on efficient, green routes and using renewable substrates. Applying the tools of synthetic biology, we design novel artificial pathways to access these chemicals. However, analyzing important target molecules with a retrosynthetic perspective we have to realize that we quite often lack enzymatic functions limiting this synthetic biochemistry approach. How do we get access to these sought-after enzymatic activities? In my talk, I will discuss grasping this challenge with a pinch from biology and chemistry.

Biologists provide us with structural information and sequence date for hundreds of closely related enzymes. Comparing these different enzymes, we can extract valuable information about structure function relationships. We can learn that nature has chosen certain hot spots and amino acid residues to adapt a protein towards a new function. Hot spots, insertions and deletions or sometimes correlated sides of amino acid variations, are an ideal starting point for designing small, focused libraries of enzyme variants. This strategy was successfully applied in engineering dioxygenases with an extended substrate spectrum and P450 monooxygenases with a higher activity.

Identifying enzyme variants which catalyze new reactions is a bit more challenging. Moon shine activities or enzyme promiscuity has thought us that enzymes can do much more than converting physiological substrates. Under the order of the same catalytic mechanisms chemically related compounds can be converted and new type of reactions are performed by the very same enzyme. Even if the reactions are running with low rates promiscuous substrates are the starting point for the development of enzymes with novel functions. Key to unravel possible reaction is the careful choice of the right substrates and calls for some chemical knowledge. This approach will be illustrated based on examples with squalene hopene cyclases catalyzing non-natural Prins-reactions, Friedel-Crafts-reaction, and isomerizations.

Insights into biological diversity of enzymes and knowledge about the diversity and mechanisms of chemical reaction enable us to engineer enzymes with novel activities in our laboratories. These new *bio*catalysts go far beyond what nature has intended to catalyze so far and expand the biosynthetic options we have quite a bit. However, we also get a notion about the evolutionary options nature has built into these fantastic catalysts.

FEMS7-1676
Cell-cell communication, signalling and quorum sensing

QUORUM SENSING VIA INTERNALIZED PEPTIDES IN STREPTOCOCCI

R. Gardan[1]
[1]*INRA, Institut Micalis- AgroParis Tech- Université Paris-Saclay, Jouy-en-josas, France*

Quorum-sensing (QS) is a bacterial cell-cell communication mechanism that controls gene expression at the population level via secreted signaling molecules. In Gram-positive bacteria, the signaling molecules are mostly small secreted peptides which are matured by a protease, actively released into the extracellular environment, accumulate and, once at a threshold concentration, are detected by a sensor protein. This sensing leads cells to modulate gene expression in a coordinated manner. For a long time, only mechanisms involving peptides that are extracellularly detected by histidine kinases of two component systems have been studied. However, a second activation pathway involving signaling molecules that need to be actively transported inside the cell before interacting with their receptors has emerged. These receptors form a structural family called RRNPP (for **R**gg/**R**ap/**N**prR/**P**lcR/**P**rgX) that is characterized by the presence of tetratrico peptide repeats (TPRs), a structural motif mediating protein-protein interactions. Members of this family are transcriptional regulators except Rap proteins that regulate the phosphorylation level or the DNA-binding activity of response regulators. RRNPP proteins regulate important physiological processes such as conjugation in enterococci (PrgX), sporulation (Rap) and virulence (PlcR) in bacilli. Rgg transcriptional regulators are found in the order of Lactobacillales and the family of Listeriacae. Up to now, they have only been studied in streptococci in which they control the triggering of competence for transformation, virulence, production of biofilm or of modified secreted peptides. In this presentation, different QS mechanisms involving Rgg regulators will be presented, compared to each other and to mechanisms involving other members of the RRNPP family, in an evolutionary perspective. We will also address the following questions: is it possible to inhibit or activate these Rgg-associated QS mechanisms and what are the issues of such manipulations?

FEMS7-0660
Cell-cell communication, signalling and quorum sensing

ANTIMICROBIAL TARGETS AND BIOMARKERS-EXPLOITING ALKYLQUINOLE-DEPENDENT QUORUM SENSING IN PSEUDOMONAS AERUGINOSA

P. Williams[1]
[1]*Centre for Biomolecular Sciences, School of Life Sciences, Nottingham, United Kingdom*

In bacterial pathogens such as *Pseudomonas aeruginosa* the expression of multiple virulence and biofilm development genes is often co-ordinately controlled at the transcriptional level by global regulatory systems incorporating quorum sensing (QS). The latter constitutes a cell-to-cell communication network that integrates information at the population level, co-ordinating the metabolic status of the cells with environmental cues. QS depends on the synthesis, secretion and perception of diffusible signalling molecules that enable pathogens to synchronize their behavior. QS signal molecules, although largely considered as effectors of QS-dependent gene expression are also emerging as multi-functional agents that impact on host-pathogen interactions and influence life, development and death in single and mixed microbial populations. QS is a potential antibacterial target in pathogens where strains carrying mutations in key QS genes exhibit highly attenuated pathogenicity in animal infection models Further indications that QS systems are active during human infections are emerging from clinical studies where QS signal molecules can been detected in patient body fluids. Since QS by definition depends on small molecule ligand/receptor interactions, it offers a direct pharmacological pathway to inhibitor development since the steric requirements for optimal ligand/receptor interactions means that antagonists can be readily obtained through structural modification of native agonists. In this context, advances in our understanding of the molecular and structural biology of alkylquinolone (AQ)-dependent QS in *P. aeruginosa* have lead to the identification of multiple 'druggable' targets and small molecule inhibitors. In cystic fibrosis, the presence of AQs in plasma and urine correlates with clinical status highlighting their potential as biomarkers to aid diagnosis and assessment of the response to treatment.

FEMS7-0059
Pathogenic yeast

CANDIDALYSIN A FUNGAL PEPTIDE TOXIN

B. Hube[1]
[1], *Germany*

The dimorphic fungus *Candida albicans* can be both, a harmless commensal of mucosal surfaces and an aggressive human pathogen in susceptible hosts. In the commensal phase, fungal cells are associated with host mucosal surfaces and co-exist with the microbiota. These conditions change during the transition to a pathogenic life style. This transition includes direct attachment to, invasion into, and damage of epithelial cells. Adhesion to host epithelial cells is mediated by surface proteins. Fungal−host surface contact during attachment can induce the production of hyphae and expression of hyphae-specific genes, which, in turn, drive further adhesion. Hyphae are not only more adhesive, but also more invasive than yeast cells. Invasion occurs via two different routes: induced endocytosis or active penetration. Most of the tissue damage is due to deep and destructive inter-epithelial invasion via elongated hyphae, along with the release of destructive factors. In fact, *C. albicans* secretes a peptide toxin, Candidalysin, which is predominantly responsible for epithelial damage.

FEMS7-0284
Pathogenic yeast

NEW INSIGHT INTO EFFECTOR FUNCTION IN THE U. MAYDIS-MAIZE PATHOSYSTEM

R. Kahmann[1], N. Ludwig[1], L. Liang[1], K. Schipper[1], S. Reissmann[1], D. Assmann[1], T. Glatter[2]
[1]Max Planck Institute for Terrestrial Microbiology, Organismic Interactions, Marburg, Germany
[2]Max Planck Institute for Terrestrial Microbiology, Proteomics Facility, Marburg, Germany

New insights into effector function in the *U. maydis*-maize pathosystem

Regine Kahmann, Nicole Ludwig, Liang Liang, Kerstin Schipper*, Stefanie Reißmann, Daniela Assmann and Timo Glatter Max Planck Institute for Terrestrial Microbiology, Dept. Organismic Interactions, Marburg, Germany

The fungus U. maydis causes smut disease in maize. U. maydis is a biotrophic pathogen requiring living plant tissue for colonization. For a successful infection U. maydis needs to suppress plant defense responses and manipulate host physiology for its own benefit. To accomplish this, U. maydis secretes a cocktail of about 300 effector proteins. The majority of these proteins lack a functional annotation and their function remains to be uncovered. Our current work focuses on effectors expressed early during infection. Systematic deletion of the most highly expressed effector genes in this class resulted in the discovery of three mutants unable to cause disease. Their mutant phenotype resembled previously identified *stp1* (stop after penetration) mutants, and the newly identified genes were designated *stp2*, *stp3* and *stp4*. A similar phenotype was also observed for mutants lacking the essential effector *pep1* (Doehlemann *et al.,*2009*). All stp mutants* strains were able to form appressoria that penetrate maize epidermal cells, but their growth was arrested in epidermal tissue. In all cases growth arrest was accompanied with the elicitation of plant defense responses and plant cell death.Co-IP with individually tagged effectors followed by mass-spectroscopic analysis revealed that Stp1, Stp3, Stp4 and Pep1 form a complex. Stp2 is not part of the complex. We will discuss our current efforts to localize the complex and to functionally characterize its components. In this context, we are testing the possibilities that the complex could aid in translocation of other effectors, function as a regulatory complex or exist to shield the activity of Avr proteins.

FEMS7-0995
Pathogenic yeast

CANDIDA ALBICANS CELL WALL: ROLES IN VIRULENCE, HOST INTERACTIONS AND DRUG SUSCEPTIBILITY

C. Munro[1]
[1], *United Kingdom*

The *Candida albicans* cell wall is a dynamic organelle, composed of chitin, β-1,3-glucan, β-1,6-glucan and mannoproteins. The echinocandin antifungal drugs target the cell wall by inhibiting β-1,3-glucan synthesis. The echinocandins provide effective therapy but sporadic breakthrough infections caused by resistant *Candida* isolates have been reported. *C. albicans* acquires echinocandin resistance through point mutations in the *FKS* target genes. In addition *C. albicans* responds to sub-MIC echinocandins by up-regulating chitin biosynthesis and expression of cell wall remodelling enzymes through activation of cell wall salvage pathways that involve PKC and calcium/calcineurin signalling. Cells with altered wall architecture are less susceptible to echinocandins *in vitro,* as well as in infection models. Other *Candida* species (with the exception of *Candida glabrata*) and *Aspergillus fumigatus* also activate compensatory wall remodelling when challenged with sub-MIC echinocandins, resulting in drug tolerance.

Substantial changes in the cell wall glycoproteome are triggered by cell wall and envelope stresses, including echinocandin treatment. Several predicted carbohydrate-active enzymes (CAZy) enzymes involved in modulating and cross-linking chitin and glucan (Phr1, Phr2, Pga4, Crh11, Utr2) are among the cell wall proteins (CWPs) positively regulated in response to cell wall damage. The MAP kinase, Mkc1, and the transcription factor, Rlm1, are important in the upregulation of these CWPs. Furthermore, clinical resistant isolates have higher levels of stress-activated CWPs, even in the absence of drug, indicating their walls are different to sensitive isolates. We are investigating the role of specific CWP genes in caspofungin susceptibility, paradoxical growth and host-pathogen interactions using overexpression and null mutant strains.

FEMS7-1686
Pathogenic yeast

FUNGAL APPRESSORIA: PLATFORMS FOR DELIVERING SMALL MOLECULE EFFECTORS

J.F. Dallery[1], E. Adelin[2], J. Ouazzani[2], R. O'Connell[1]
[1]INRA, BIOGER, Thiverval-Grignon, France
[2]CNRS, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France

Species of the fungal genus *Colletotrichum* cause devastating diseases on numerous major crop plants worldwide. *C. higginsianum* attacks cultivated members of the Brassicaceae but also the model plant *Arabidopsis*. Initial host cell invasion requires the differentiation of a specialized cell-type called an appressorium that mediates penetration of the plant cuticle and cell wall. Compared to other sequenced ascomycetes, the genome of *C. higginsianum* contains an exceptionally large number of genes (89) encoding secondary metabolism (SM) key enzymes. These are organized into 68 gene clusters which, as in other fungi, also contain genes encoding accessory enzymes of the same biosynthetic pathway, together with efflux transporters and pathway-specific transcription factors. A remarkable finding from transcriptome profiling was that 19 SM clusters are specifically expressed only at early stages of plant infection inside appressoria. Since each cluster potentially synthesizes one metabolite, this suggests fungal appressoria deliver a cocktail of different chemicals to the first infected host cell. At this early stage host cells remain alive, raising the possibility that these molecules function similar to protein effectors for host manipulation, rather than as simple phytotoxins. We present evidence that the coordinated and stage-specific expression of SM genes is orchestrated at two levels, namely remodelling of chromatin structure and regulation by global transcriptional activators. Progress towards the functional characterization of these metabolites will also be presented, including evidence that some metabolites can subvert plant immune responses.

FEMS7-3303
Microbiome pathogens-host: Triangulating host-pathogen interaction in microbial infections

MICROBIOME AND CLOSTRIDIUM DIFFICILE INFECTION

H. Browne[1], S. Forster[1], T. Lawley[1]
[1]Wellcome Trust Sanger Institute, Host-microbiota interactions, Cambridge, United Kingdom

Our intestinal microbiota harbours a diverse bacterial community required for our health, sustenance and wellbeing. Intestinal colonization begins at birth and climaxes with the acquisition of two dominant groups of strict anaerobic bacteria belonging to the Firmicutes and Bacteroidetes phyla. Culture-independent, genomic approaches have transformed our understanding of the role of the human microbiome in health and many diseases. However, owing to the prevailing perception that our indigenous bacteria are largely recalcitrant to culture, many of their functions and phenotypes remain unknown. Here we describe a novel workflow based on targeted phenotypic culturing linked to large-scale whole-genome sequencing, phylogenetic analysis and computational modelling that demonstrates that a substantial proportion of the intestinal bacteria are culturable. Applying this approach to healthy individuals, we isolated 137 bacterial species from characterized and candidate novel families, genera and species that were archived as pure cultures. Whole-genome and metagenomic sequencing, combined with computational and phenotypic analysis, suggests that at least 50-60% of the bacterial genera from the intestinal microbiota of a healthy individual produce resilient spores, specialized for host-to-host transmission. Our approach unlocks the human intestinal microbiota for phenotypic analysis and reveals how a marked proportion of oxygen-sensitive intestinal bacteria can be transmitted between individuals, affecting microbiota heritability.

FEMS7-0179
Microbiome pathogens-host: Triangulating host-pathogen interaction in microbial infections

SHIGELLA, TYPE 6 SECRETION SYSTEM AND THE MICROBIOME

P. Sansonetti[1]
[1], *Paris, France*

FEMS 2017 Congress

Session name : Microbiome pathogens-host : triangulating host-pathogen interaction microbial infections

Monday July 10, 2017 (10 :00-12 :30)

Mark Anderson, Pascale Vonaesch, Azadeh Saffarian, Benoît Marteyn, **Philippe J Sansonetti**

Unité de Pathogénie Microbienne Moléculaire & Unité INSERM 2020, Institut Pasteur ; Chaire de Microbiologie et Maladies Infectieuses, Collège de France

Title : Shigella, type 6 secretion system and the microbiome

Abstract : « Cellular microbiology » has clearly biased studies on microbial pathogenesis towards focused molecular and cellular analysis of the cross-talks established between the pathogen and its target cells. « Tissue microbiology » has attempted to integrate these mechanisms into the more global scheme of the pathogen interacting with cells in their relevant context. These approaches have successfully driven the field and promoted pathogenesis research to an extraordinary degree of cellular and subcellular resolution ; they have also somewhat ignored important components of the entire pathogenic cycle such as the phases of mucosal colonisation, dissemination and transmission. Recent emergence of the microbiota on the « pathogenesis agenda » has reestablished the colonisation phase as a central issue in pathogenesis, particularly due to the need for the pathogen to secure its niche at the mucosal surface to the expence of the résident microbiota that imposes a strong barrier effect. We have developed dedicated tools to study the Shigella-microbiota-host « ménage à trois », particularly rodents models of colon infection. On these bases, we characterized two systems that secure colonization of the colonic mucosa by *Shigella sonnei* : a type 6 secretory system and a colicin that are unique to this serotype and essential to occupy the niche normally dedicated to commensal proteobacteria such as *Escherichia coli*. In addition, S. sonnei shows strong capacity to efface S. flexneri, both in vitro and in vivo, possibly explaining the current emergence of *S. sonnei* among the various Shigella serotypes that used to cause bacillary dysentery.

FEMS7-3301
Microbiome pathogens-host: Triangulating host-pathogen interaction in microbial infections

MICROBIOME AND SALMONELLA INFECTION

B. Stecher[1]
[1]*Max von Pettenkofer Institute- LMU Munich, Medicine, Munich, Germany*

A toolbox of cultured bacteria to probe the role of individual microbiota members in preventing pathogen infections

"Meta-omics" analyses are dramatically expanding our knowledge about the composition and metabolic diversity of the gut microbiota and its association with human diseases, but dissecting the function and quantitative impact of individual microbiota members remains a challenge. Protection against enteric infections, also termed colonization resistance (CR), results from mutualistic interactions of the host and its indigenous microbes. The gut microbiota of humans and mice is highly diverse and it is therefore challenging to assign specific properties to its individual members. We assembled a collection of murine bacterial strains, termed Oligo-Mouse-Microbiota and a modular design approach to create a minimal bacterial community of 12 strains that stably colonizes germ-free mice. This community provides intermediate resistance against infections with different enteric pathogens, including *Salmonella enterica* serovar Typhimurium (S. Tm) and *Clostridium difficile*. This particular phenotype allowed us to investigate, whether additional bacteria could further enhance resistance to the level of a complex, undefined microbiota. With the aid of comparative metagenomics, we created an improved version of the Oligo-MM community harboring three additional strains from a novel public culture collection (1) that provided conventional-like CR against S. Tm (2). Furthermore, supplementation of secondary bile acid producers was protective against *C. difficile* induced disease (3). In conclusion, the Oligo-MM is a highly versatile experimental system to functionally probe the role of individual microbiota members in different enteric infection models and beyond. Thus, in combination with exhaustive bacterial strain collections and systems-based approaches, gnotobiotic models can be used to generate novel insights into microbe-microbe and microbe-host interactions for the investigation of ecological and disease-relevant mechanisms in the intestine.

1. Lagkouvardos et al., *The Mouse Intestinal Bacterial Collection (miBC) provides host-specific insight into cultured diversity and functional potential of the gut microbiota.* Nat Microbiol, 2016. 1(10): p. 16131.
2. Brugiroux et al., *Genome-guided design of a defined mouse microbiota that confers colonization resistance against Salmonella enterica serovar Typhimurium.* Nature Microbiology, 2016.
3. Studer et al., *Functional Intestinal Bile Acid 7alpha-Dehydroxylation by Clostridium scindens Associated with Protection from Clostridium difficile Infection in a Gnotobiotic Mouse Model.* Front Cell Infect Microbiol, 2016. 6: p. 191.

FEMS7-0123
Decrypting microbe-cell cross-talks in the intestinal crypt: Microbiome and epithelial regenerations

DECRYPTING MICROBE-CELL CROSS-TALKS IN THE INTESTINAL CRYPT: MICROBIOME AND EPITHELIAL REGENERATIONS

P. Sansonetti[1]
[1], *Paris, France*

FEMS 2017 Congress

Session name : Invited presentation

Wednesday July 12, 2017 (09 :00-10 :000)

Giulia Nigro, Thierry Pédron, Aline Stedman, Céline Mulet, **Philippe J Sansonetti**

Unité de Pathogénie Microbienne Moléculaire & Unité INSERM 2020, Institut Pasteur ; Chaire de Microbiologie et Maladies Infectieuses, Collège de France

Title : Decrypting microbe-cell cross-talks in the intestinal crypt : microbiome and epithelial regeneration

Abstract : We have identified a limited subset of bacterial taxa called crypt-specific core microbiota (CSCM) that dwells in intestinal crypts of the caecum and colon in mice and more recently in humans. These bacteria share common metabolic properties, being strictly aerobic and non fermentative. Initially identified by metataxonomics, they have been more recently isolated and cultivated and belong to three dominant genus : Acinetobacter, Delftia and Stenotrophomonas. Intestinal crypts are sites of sustained epithelial regeneration and repair in case of injury. Intestinal stem cells are located at the bottom of the crypts, thus in direct contact with stem cells and their proliferating/differentiating lineages. The intestinal crypt thus appears like a good model to study the impact of the microbiota on tissue regeneration and repair. We have identified a MDP-Nod2 signaling pathway that provides cytoprotective capacities to stem cells upon agression by a cytotoxic drug such as Doxorubicin. Studies are on their way to decipher the relevant mechanisms of protection. Focusing on CSCM components, we have shown that they achieve a balance between controled prolifération and stimulated differentiation in a LipidA-TLR4-dependant pathway. Hence the cytoprotective and homeostatic role of CSCM is becoming central to this topic.

FEMS7-2928
In search of thermodynamic principles for managing microbial communities in environmental biotechnology processes

IN SEARCH OF THERMODYNAMIC PRINCIPLES FOR MANAGING MICROBIAL COMMUNITIES IN ENVIRONMENTAL BIOTECHNOLOGY PROCESSES

T. Bouchez[1], H. Delattre[1], E. Desmond-Le Quéméner[1]
[1]Irstea, HBAN, Antony, France

Environmental microbial communities are key engines that drive earth's biogeochemical cycles. Despite their enormous diversity, microbiomes exhibit a remarkable property: the functional convergence. It characterizes their general tendency to reproduce defined functional population patterns in specific biotopes (sea water, soil, plant foliage, cheese, human body biomes...). In environmental biotechnology processes such as wastewater treatment plants or anaerobic digesters, this property allows to maintain and exploit functionally defined but taxonomically diverse mixed microbial cultures in open systems by applying selective pressures. This suggests that, beside random population immigration/emigration events, mechanistic processes largely determine functional microbial community assembly patterns. Here, we examine the contribution of energy gradients to the emergence of these patterns.

We build on a recent thermodynamic theory of microbial growth in which flux/force relationship between functional population growth rates and available energy gradients is derived from statistical physics reasoning. Coupled to mass and energy balance calculations, this relationship is used to simulate the dynamics of virtual microbial ecosystems. Simulations show that consistent ecological successions and functional community patterns arise from the model without population specific parameter calibration. Applied to activated sludge modelling, virtual communities exhibit emergent behaviours consistent with current engineering knowledge. Our approach shows how chemical species in solution determine the metabolic niches that can sustain growth, from which functionally convergent microbial community patterns arise. In the future, this approach could be used to build ecological models coupling energy balance, stoichiometry and microbial dynamics for better managing the functional abilities of microbial communities.

FEMS7-0722
Innovators and imitators: how environmental bacteria conquest the chemical space

INNOVATORS AND IMITATORS: HOW ENVIRONMENTAL BACTERIA CONQUEST THE CHEMICAL SPACE

Ö. Akkaya[1], D. Perez-Pantoja[2], P.I. Nikel[3], V. de Lorenzo[4]
[1]Gebze Technical University, Molecular Biology and Genetics, Kocaeli, Turkey
[2]Programa Institucional de Fomento a la I+D+i PIDi, Universidad Tecnológica Metropolitana, Santiago, Chile
[3]The Novo Nordisk Foundation Center for Biosustainability, Cell Factories, Lyngby, Denmark
[4]Centro Nacional de Biotecnología, Systems Biology, Madrid-Campus de Cantoblanco, Spain

The still-evolving 2,4-dinitrotoluene (DNT) pathway of *Burkholderia cepacia* R34 has been studied as a case of emergence of new metabolic capabilities in environmental bacteria. The *dnt* route originated from a precursor naphthalene degradation pathway and the first enzyme (DNT dioxygenase) maintains significant activity towards its earlier substrate. Both *in vivo* reactions and the associated regulatory system mediated by the DntR transcriptional factor indicate that reactive oxygen species (ROS) generated by the faulty (i.e. uncoupled) reaction of the precursor enzymes with DNT elicit genetic diversification. This could in turn ease the solution of the biochemical and physiological problem. When the *dnt* system was transplanted to the genetically tractable background of *Escherichia coli*, mutagenesis caused by endogenously produced ROS was dependent on *rpoS* and *dinB,* and was not accompanied by a general induction of the SOS response. In addition, analysis of the type of mutations suggested that ROS-triggered genetic diversification was due not so much to misincorporation of 8-oxoguanine as to the lack of fidelity of DNA replication. When the *dnt* operon was inserted in the genome of *Pseudomonas putida* and cells were exposed to DNT, the resulting metabolic ROS did not translate in significantly higher mutagenic rates. Artificially decreasing the intracellular pool of NAD(P)H caused *P. putida dnt*+ to acquire a high genetic-diversification regime. It is thus plausible that some members of a given microbial community are prone to innovate their metabolic capacities much faster than others while the rest may benefit from such innovation through horizontal gene transfer.

FEMS7-2927
Lwoff-Award Lecture

CELL WALL DEFICIENT (L-FORM) BACTERIA: FROM CHRONIC INFECTIONS TO THE ORIGINS OF LIFE

Y. Kawai[1], K. Mickiewicz[1], J. Errington[1]
[1]Newcastle University, Centre for Bacterial Cell Biology, Newcastle-upon-Tyne, United Kingdom

The peptidoglycan cell wall is a defining structure of the bacteria. It is the target for our best antibiotics and fragments of the wall trigger powerful innate immune responses against infection. The genes for peptidoglycan synthesis are present in most bacterial lineages, suggesting that the wall emerged early in cellular evolution. Surprisingly, many bacteria can switch almost effortlessly into a cell wall deficient “L-form” state in which they become completely resistant to many cell wall active antibiotics. Remarkably, L-form growth is completely independent of the complex FtsZ-based division machine that is essential for proliferation of most bacteria. Proliferation occurs instead by a seemingly haphazard process involving membrane blebbing or tubulation and scission, leading to progeny of highly irregular size. The switch to this mode of proliferation seems to require only the upregulation of membrane synthesis, leading to an increased surface area to volume ratio. L-forms may provide insights into how primitive cells proliferated before the evolution of the cell wall and the ancient bacterial radiation. L-forms have also been implicated in many recurrent or chronic diseases, although these claims have been controversial. Recent results uncovering an unexpected interplay between the action of β-lactam antibiotics, endogenous lysozyme and L-form switching, which may be highly relevant to the treatment of infectious diseases, will be described.

Key references

Leaver et al., 2009, **Nature** 457, 849-853. Mercier et al., 2013, **Cell** 152, 997-1007. Errington, 2013, **Open Biology** 3, 120143. Mercier et al., 2014, **eLife** 04629. Kawai et al., 2015, **Current Biology** 25, 1613-1618; Mercier et al., 2016, **Nature Microbiology** 1, 16091. Errington et al., 2016, **Philos Trans R Soc Lond B Biol Sci**. 371, 20150494.

FEMS7-0460
Virology, new viruses, RNA polymerase

VIROLOGY, NEW VIRUSSES, RNA POLYMERASE

S. Cusack[1]
[1], *France*

The 'flu is caused by the highly infectious, rapidly evolving and potentially dangerous influenza virus. We study the molecular mechanisms by which the viral RNA-dependent RNA polymerase transcribes and replicates the viral genome. This is not only of fundamental interest but will also help understand avian to human interspecies transmission of the virus and promote development of new anti-influenza drugs. Influenza, which is a member of the *Orthomyxoviridae* family of segmented negative strand RNA viruses (sNSV), continues to have an enormous impact on world-wide public health. The eight distinct genome segments are individually packaged into ribonucleoprotein particles (RNPs), which are the functional replication units. Transcription, generating capped and poly-adenylated viral mRNAs, and replication, generating full-length genome or antigenome copies (vRNA and cRNA respectively), are performed by the same virally encoded RNA-dependent RNA polymerase. In general, sNSV polymerases have two unique features. Firstly, they perform transcription by the 'cap-snatching' mechanism, whereby short 5′ capped RNA fragments are cleaved from host cell mRNA by an endonuclease intrinsic to the polymerase and then used to prime synthesis of viral mRNAs. Secondly, they recognise each genome segment via their highly conserved, quasi-complementary 3′ and 5′ extremities, known as the promoter. Several recent crystal structures of promoter bound influenza A and B polymerases that shed light on multiple aspects of polymerase function will be discussed and their implication for novel anti-viral drug design targeting directly viral replication highlighted. Most recently we have shown that to gain access to nascent capped transcripts for 'cap-snatching' in the infected cell nucleus, influenza polymerase directly binds the phosphorylated C-terminal domain of cellular RNA polymerase II.

References

1. **Structure of influenza A polymerase bound to the viral RNA promoter**. Pflug, A. *et al*. Nature. 2014.

2. **Structural insight into cap-snatching and RNA synthesis by influenza polymerase**. Reich, S. *et al*. Nature. 2014.

3. **Structural insights into bunyavirus replication and its regulation by the vRNA promoter**. Gerlach, P. *et al*. Cell. 2015.

4. **Influenza polymerase can adopt an alternative configuration involving a radical repacking of PB2 domains**. Thierry, E. *et al*. Mol Cell. 2016.

5. **Structural analysis of specific metal chelating inhibitor binding to the endonuclease domain of influenza pH1N1 (2009) polymerase**. Kowalinski, E. *et al*. PLoS Pathog. 2012.

6. **Structural basis for an essential interaction between influenza polymerase and Pol II C-terminal domain**. Lukarska, M. *et al*. Nature 2017.

FEMS7-0897
Bacterial RNA Secretome

DEVELOPMENT OF AN AGAR-BASED RNA-SEQ ASSAY FOR STUDYING THE INTERACTOME OF NASAL STAPHYLOCOCCI STRAINS

G. Hermansen[1], *L. Jelsbak*[1]
[1]*Technical University of Denmark, DTU Bioengineering, Kgs Lyngby, Denmark*

Backgrounds

Staphylococcus aureus is well-known as a human pathogen but also lives as a commensal in the human nose in approximately one fifth of the population. Carriage is usually asymptomatic but the nasal cavities can function as reservoirs for infection e.g. during surgical procedures. The human nose hosts a multi-species community and it has previously been reported that *Staphylococcus epidermidis* strains can modulate *S. aureus* phenotypes. Intriguingly the cohabitation has been shown to result in inhibition of nasal colonization and biofilm formation of *S. aureus*. Even though some *S. epidermidis* products have been implicated in this inhibitory effect, a comprehensive understanding of the interactions between nasal Staphylococci isolates is needed.

Objectives

The aim of this study was to establish a robust method to map the transcriptome profiles of nasal *S. aureus* and *S. epidermidis* focusing on the differences between mono- and co-culturing.

Methods

Staphylococci strains were isolated from nasal cavities of healthy Danish individuals. A pair of *S. aureus* and *S. epidermidis* strains isolated from the same nose were used for a novel agar-based RNA-seq method enabling investigation of their interactome. The strains were grown on agar surfaces as mono- and co-cultures, respectively, and RNA was harvested after 24 hours of incubation. RNA-seq was performed and enables mapping of the expression profiles of the two nasal Staphylococci isolates.

Conclusions

Our preliminary results indicate this novel RNA-seq method can be applied to study interactions between *S. aureus* and *S. epidermidis* isolated from nasal swaps of a healthy Danish adult.

FEMS7-2052
Bacterial RNA Secretome

QUORUM SENSING BASED ON INTERSPECIES COMMUNICATION AMONG E. COLI SE15 AND OTHER STRAINS OF BIOFLM IN THE PATIENTS INDWELLING URINARY CATHETERS: GENOME EDITING OF CRISPR-CAS9

S.R. Kang[1], J. Kim[2], J.K. Hur[3], S.S. Lee[1]
[1]Kyonggi University, Department of Bio-engineering, Suwon, Republic of Korea
[2]Kyonggi University, Department of Life Science, Suwon, Republic of Korea
[3]Korea University, Biomedical Research Center- Anam Hospital, Seoul, Republic of Korea

Backgrounds

The predominant strains *E. coli*, *P. aeruginosa* and *S. aureus* are the most common pathogens in catheter associated urinary tract infections (CA UTIs). The CA UTIs were mainly occurred from biofilm formation by Quorum Sensing (QS) system of predominant strains. Moreover, AI-2, one of QS signal molecules, plays an important role in enabling interspecies communication. Although many bacteria species have been studied to use the AI-2 for interspecies communication to regulate various behaviors, such as biofilm formation, the molecular mechanisms of AI-2 internalization and signal transduction remain poorly understood.

Objectives

This study is to elucidate interaction between *E. coli* SE15 and other dominant strains, which frequently form biofilm on the catheter surface, via co-culture.

Methods

We then manipulated the Δ*rbsB* and Δ*luxS* in *E. coli* SE15 by using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system. And we observed biofilm formation assay in Δ*rbsB* and Δ*luxS* mutants of *E. coli* SE15. Moreover, Δ*rbsB* and Δ*luxS* mutants of *E. coli* SE15 were co-cultured with predominant strains *P. aeruginosa* and *S. aureus* in response to the wild type *E. coli* and analyzed AI-related genes expression by qRT-PCR.

Conclusions

Autoinducer synthesis gene *luxS* gene of *E. coli* SE15 has been affected by other dominant strains because the gene expression was more increased and rapidly initiated in co-culture than single culture. Based on these data, we concluded that *luxS* and *rbsB* affected on interaction of other predominant bacteria. Such interactions among predominant bacteria were helpful to understand their symbiosis or competition.

FEMS7-2642
Bacterial RNA Secretome

TRANSCRIPTOME AND SECRETOME OF SHORT RNAS IN BACTERIAL POPULATIONS

O. Ozoline[1], *A. Bykov*[1], *K. Shavkunov*[1]
[1]*Institute of Cell Biophysics RAS, Functional Genomics and Cellular Stress, Pushchino, Russia*

Backgrounds

Switching research from individual genes to large-scale analysis of transcriptomes revealed a huge amount of untranslated RNAs with poorly understood functions. The least studied are short RNAs, including those processed from tRNAs or produced from gene ends, though their regulatory potential has been recently elucidated.

Objectives

The goal of the study was to characterize the intracellular and extracellular fractions of short (11-14 nucleotides) RNAs, in particularly those derived from “promoter islands” suppressed by H-NS.

Methods

Fractions of short RNAs from cells of *E. coli* K-12 MG1655 and novel isolate of the *Paenibacillus* genus were obtained using mirVana™ miRNA Isolation Kit. Short secreted RNAs were purified from filtered LB medium using miRNeasy Serum/Plasma Kit (Qiagen). cDNA libraries were prepared using Ion Total RNA-Seq Kit v2 and sequenced with Ion Torrent PGM. Sequence reads were mapped to the genomes with Matcher algorithm.

Conclusions

In both bacteria intracellular fractions of short RNAs mainly contained fragments of tRNAs, rRNAs and small untranslated RNAs. The proportion of RNAs derived from the “promoter islands” of *E. coli* was lower than on average over the genome; however, their abundance in the extracellular fraction was twice higher. The distribution profile of the secreted RNAs across the genomes differed, as compared to their intracellular RNAs. Cooperative culturing of two microorganisms allowed detecting evident divergence between the sets of extracellular RNAs in pure and mixed cultures. Bacteria, therefore, secrete not only proteins and small signaling molecules, but also short RNAs, which may participate in cell-to-cell communications.

FEMS7-0407
Bacterial RNA Secretome

OPTIMAL RNA EXTRACTION METHOD FOR THE ANALYSIS OF BACTERIAL SMALL RNAS CONTAINED IN MEMBRANE VESICLES

C. Garcia-Bonillo[1], C. Perez-Cruz[1], E. Mercade[1]
[1]University of Barcelona, Department of Biology- Healthcare and the Environment, Barcelona, Spain

Backgrounds

Membrane vesicles (MVs) are released by many Gram-negative bacteria and *Pseudomonas aeruginosa* is one of them. Currently, the presence of *small* RNA (sRNAs) in MVs is being extensively analysed in eukaryotes but remains nearly unexplored in prokaryotes. The methodology of extraction of sRNAs may influence the results obtained in further studies.

Objectives

To determine the optimal RNA extraction method for the analysis of bacterial *s*RNAs contained in MVs in order to support future studies aimed at determining the biological roles of these sRNAs.

Methods

Three different RNA extraction methods were assayed: miRCURY™ RNA isolation kit – Cell & Plant content (EXIQON®), FastRNA™ SPIN Kit for Microbes (MP®) and miRNeasy Mini Kit (QIAGEN®). These kits were used to extract total RNA and, quality and purity of extracted RNA was determined by OD values, capillary electrophoresis and fluorimetry. The efficiency of extraction of *P. aeruginosa* PAO1 *s*RNAs (RsmZ, CrcZ and PhrS) was studied by RT-qPCR.

Conclusions

Results indicate that the kits EXIQON® and MP® extract more effectively the *small* RNAs fraction than QIAGEN®. EXIQON® is recommended to extract *small* RNAs from MVs because the obtained RNA profiles are cleaner, the protocol is easier to perform and the number of copies does not present significant differences with MP®. As the different RNA isolations methods give extensive variations in the sRNAs yields and patterns, it is crucial to select an RNA isolation approach depending on the research purpose.

FEMS7-2467
Bacterial RNA Secretome

BACTERIAL TRANSCRIPTOME REPROGRAMMING IN NODULES OF PEA (PISUM SATIVUM L.) SYMBIOTIC MUTANTS

V. Zhukov[1], A. Afonin[1], A. Sulima[1], A. Zhernakov[1], I. Tikhonovich[1]
[1]All-Russia Research Institute for Agricultural Microbiology, Biotechnology, Saint-Petersburg - Pushkin, Russia

Backgrounds

Nodule bacteria can fix atmospheric nitrogen in symbiosis with legume plants. In nodules of some legumes, including pea (*Pisum sativum* L.), bacteria are terminally differentiated into organelle-like structures called bacteroids. The process of terminal bacteroid differentiation (TBD) implies cell enlargement, genome amplification and membrane permeabilization. Mutations in plant regulatory symbiotic genes may lead to defects in TBD. Analysis of such model systems using transcriptomics approach can help unravel the molecular bases of TBD.

Objectives

To describe the influence of plant mutations on expression of bacterial genes in case of abnormal TBD and to find possible new factors and components playing a role in this process.

Methods

To assess gene expression levels, we used 5'-MACE (Massive Analysis of cDNA Ends) technology developed by GenXPro GmbH (Frankfurt-am-Main, Germany), which involves sequencing of 5'-part of each transcript on Illumina HiSeq2000. The total RNA was extracted from the following sources: i) free-living culture of *Rhizobium legominosarum* bv. *viciae* RCAM1026, ii) nodules of wild type pea line SGE, iii) set of mutants in pea genes *sym33*, *sym40* and *sym26* obtained on SGE background, which demonstrate block on sequential stages of TBD.

Conclusions

Bacterial genes crucial to cooperative metabolism of micro- and macrosymbiont were identified, along with a number of multi-drug resistance genes possibly modulating the effects of NCR peptides (plant anti-microbial agents promoting TBD) in nodules.

This work was supported by Russian Science Foundation [grant 14-24-00135 for AAM, ZAI, TIA and grant 16-16-00118 for ZVA, SAS].

FEMS7-3167
Environmental Microbiology

ECOLOGICAL INTERPLAYS IN THE TRANSITION TO MULTICELLULARITY

B. Baselga Cervera[1], *M. Travisano*[2]
[1]*Universidad Complutense de Madrid, Animal Production, MAdrid, Spain*
[2]*University of Minnesota, College of Biological Sciences, st. Paul, USA*

Backgrounds

Throughout the history of life, the evolution from single-celled to pluricellular organisms emerged, somehow serendipity, more than 20 times under separate scenarios. This mayor evolutionary transition constituted a source of novelty that reshaped the Earth's biodiversity and ecology. Notwithstanding recent discoveries both experimentally and in the fossil record, the underling processes and mechanisms of multicellularity arise are poorly understood.

Objectives

The complex interplays between organisms and their environments, lead to multiple interactions, features and properties. Thus, ecological conditions might have played a significant role during the transition to multicellularity and it persistence. Here, we examined the implications of different environmental conditions, related with resources availability, in the origin of multicellularity from an ecological perspective.

Methods

By means of experimental evolution and capitalizing the advantages of using the model organisms *Saccharomyces cerevisiae*, we have performed two different 2x2 factors experiments based on the original experiment of Ratcliff et al. (2012). Each experiment presented two types of environmental conditions: YPD rich media and Minimal media, and two types of treatment; no selection and settling selection, starting from differentness ancestral populations: a unicellular *S. cerevisiae* strain (Y55) and an evolved multicellular strain, snowflake yeast strain.

Conclusions

The evolution from unicellular to multicellular phenotypes took place in minimal media under setting selection, however we found different phenotypic distributions among replicates. The recently evolved snowflake yeast phenotype strain did not return to its ancestral unicellular phenotype in any of the environmental conditions. Ecological differences can lead to different results in the evolution of multicellularity.

FEMS7-1321
Environmental Microbiology

CONSISTENT DYNAMICS AND COMMUNITY ASSEMBLY PATTERNS DIRECTLY ARISING FROM THERMODYNAMIC THEORY OF MICROBIAL GROWTH

H. Delattre[1]
[1]*IRSTEA, HBAN, antony, France*

Backgrounds

Microbial communities are key engines that drive earth biogeochemical cycles. Developing models able to capture and predict their dynamics and community assembly patterns is therefore of outmost importance for the study of global earth ecological equilibria and the development of innovative microbial biotechnology processes. However, ecosystem models today exhibit only limited abilities in predicting microbial dynamics and require the calibration of multiple population specific empirical equations.

Objectives

In order to build more generic models, there is a need to more thoroughly capture the fundamental drivers of microbial growth and to mathematically express how they contribute to the emergence of the many community assembly patterns observed in nature.

Methods

We simulate the dynamics of microbial functional communities using a kinetic model of microbial growth based on statistical physics (Desmond-Le Quéméner & Bouchez, 2014). We show how the theory coupled to simple mass and energy balance calculations offer a parsimonious explanation for many well-known microbial community assembly patterns along redox gradients.

Conclusions

Strikingly, considering only these simple physical assumptions, we show that consistent microbial dynamics, successions and functional community assembly patterns can be obtained, without population-specific parameter calibration, for systems ranging from pure cultures to communities of multiple populations.

FEMS7-1297
Environmental Microbiology

HOW DO MARINE BACTERIA INTERACT WITH THE AMOEBA ACANTHAMOEBA CASTELLANII ?

R. Guillonneau[1], *C. Baraquet*[1], *M. Molmeret*[1]
[1]*Université de Toulon, MAPIEM EA 4323, La Garde, France*

Backgrounds

Amoebae are common in soils, freshwater and marine ecosystems. They are suspected to have an important role in most microbial ecosystems, especially in biofilm. However the interaction between these protozoa and marine bacteria is poorly investigated

Objectives

The aim of this work is first to study interactions between different marine bacteria and the axenical amoeba *Acanthamoeba castellanii* in order to identify different behavioural patterns. The second objective is to understand if, in this context, the bacterial biofilms could provide any sort of advantage against this predator.

Methods

Bacteria-specific antibodies were used during the co-culture experiments for confocal laser scanning microscopy observations while amoebas were labelled with DAPI. Transmission electronic microscopy was also used to visualize intracellular localisation of bacteria.

Conclusions

These marine bacteria presented different profiles of interaction with *Acanthamoeba castellanii.* They appeared to be localized in different intracellular compartments or to behave in a singular manner. For instance, *Persicivirga mediterranea* (TC4) was located in the amoeba nucleus, which could at least in part protects the bacteria from phagolysosomal digestion. *Shewanella sp.* (TC11) appears to be within suspected expelled fecal balls after its interaction with the amoeba. The objective was to identify the different bacterial behaviour within the host cell, and to understand if these behaviours could trigger specific outcomes when the protozoa is added to monospecific and multispecies biofilms. This is one of the first study comparing different isolated marine bacterial species in presence of the amoeba *A. castellanii*.

FEMS7-2724
Environmental Microbiology

THE MICROBIAL ECOSYSTEM OF THE GREENLAND ICE SHEET SURFACE

C.S. Jacobsen[1], K. Cameron[2], S. Marek[3]
[1]Aarhus University, Environmental Sciences, Roskilde, Denmark
[2]Geological Survey of Denmark and Greenland, Department of Geochemistry, Copenhagen, Denmark
[3]Charles University, Department of Ecology, Pragh, Czech Republic

Backgrounds

The surface of the Greenland ice sheet (GrIS) harbors a simple yet unique microbial ecosystem. The biological active margin of the ice sheet is more than 200 000 km^2, can be up to ~100km wide, and has tended to increase in area each year within recent history. This ecosystem contains microbial communities that are important for biogeochemical processes and surface melt and that are likely important for the injection of biota and nutrients into downstream environments.

Objectives

Our research suggests that the GrIS surface receives a “baseline” cell supply via deposition of atmospheric waters, and that wind-borne dust deposited on the ice sheet likely contains additional cells and may provide limiting nutrients for microbial growth. Ablation areas with high dust concentrations and longer melt seasons are therefore expected to contain higher numbers of active microbes compared to the accumulation area.

Methods

Geochemical anlysis, qPCR and amplicon seqiencing.

Conclusions

Within the ablation area, significant differences exist between the bulk and potentially active bacterial communities collected at the margin and the interior of the GrIS, with a higher total abundance but lower proportion of active bacteria at the margin. Higher microbial activity in the interior compared to the margin is also supported by productivity measurements. Nitrate had no significant effect on the abundance and community structure, which suggests that this system is not nitrate limited. In terms of diversity, we do not find a core, uniform bacterial community across the biologically active area of the GrIS; instead, km-scale variations between the microbial communities exist.

FEMS7-1441
Environmental Microbiology

MONITORING BACTERIAL DEPOSITION IN POROUS MEDIA BY PREDATORY BACTERIA

R. Sungthong[1], *W. T. Sloan*[1], *A. J. Pinto*[2]
[1]*University of Glasgow, School of Engineering, Glasgow, United Kingdom*
[2]*Northeastern University, Department of Civil and Environmental Engineering, Boston- MA, USA*

Backgrounds

Bacterial deposition in porous media is an important microbial property affecting range of environmental technologies, e.g., prevention of pathogen dispersion towards groundwater, optimization of subsurface bioremediation, and development of biofiltration in water treatments.

Objectives

In order to optimize the biofiltration by exploiting predatory bacteria, we assessed how these bacteria behave through porous media of sand-packed columns and how they influence the transport and deposition of a coliform bacterium, *Escherichia coli* through the columns.

Methods

Bdellovibrio bacteriovorus HD100 and its saprophytic mutant (HI100) were utilized as predatory bacteria.

Conclusions

Our findings revealed that only ~20% of HD100 cells could deposit in the columns, and such amount of deposited HD100 cells could diminish the deposition rate of *E*. *coli* cells in the same columns by up to 35%. However, up to 55% of *E*. *coli* cells pre-deposited in the columns was removed after dosing with HD100 cell suspension. Although pre-saturated cell-free culture (CFC) broth of strain HI100 in the columns did not show significant effect on the deposition rate of *E*. *coli* cells, it could remove up to 50% of *E*. *coli* cells pre-deposited in the columns. The viability of *E*. *coli* in the column effluents did not change after dosing with HD100 cell suspension or CFC broth of strain HI100, suggesting that either deposition or detachment of *E*. *coli* cells was a result of the interfacial interactions with predatory bacteria and/or their extracellular metabolites. This study provides new insights into an attempt for implementing microbial management in the optimization of biofiltration design and performance.

FEMS7-0252
Environmental Microbiology

HOW TO TRANSMIT A GUT BACTERIAL SYMBIONT IN SOCIAL INSECTS?

M. Zhukova[1], *P. Sapountzis*[1], *M. Schiøtt*[1], *J.J. Boomsma*[1]
[1]*University of Copenhagen, Centre for Social Evolution- Department of Biology, Copenhagen, Denmark*

Backgrounds

The transmission of bacterial symbionts across host generations is crucial for the direction and efficiency of host-symbiont coevolution. Specialized gut microbes of ants are peculiar because within-colony horizontal transmission can produce exclusive vertical transmission across generations. Colony life may thus alleviate constraints in the pupal stage where direct transmission from larval- to adult guts is impossible, particularly when the pupal environment is predictable.

Objectives

We analysed gut microbiome changes across developmental stages and traced the bacterial transmission routes of two abundant gut symbionts of *Acromyrmex* and *Atta* leafcutter ants, which are important functional herbivores in the New World tropics.

Methods

We analysed Illumina MiSeq 16S rRNA gene amplicon sequencing data to determine gut bacterial communities at different developmental stages. We also measured bacterial abundance using droplet digital PCR, and used confocal and electron microscopy to visualize bacteria in different gut tissues.

Conclusions

The larval and pupal guts contained several bacterial species that also occur in the fungus gardens, while adult workers hardly have these bacteria in spite of eating the same fungal material. Non-parasitic *Wolbachia* were most abundant across developmental stages in *Acromyrmex*, but *Atta* had mostly Mollicutes in the worker guts. *Wolbachia* appears to be transovarially transmitted, but Mollicutes were absent in eggs and scarce/absent in larval and pupal guts. After eclosion, callows acquired Mollicutes through interactions with other workers and failed to obtain these bacteria in isolation, suggesting that Mollicutes are socially transmitted. Both pathways result in exclusive vertical transmission across generations and should therefore facilitate host-symbiont coevolution in similar ways.

FEMS7-3104
Food Microbiology, omics driven molecular physiology

EFFECT OF (SUB)LETHAL LEVELS OF FOOD-RELATED STRESSES (BIOCIDES AND PROCESSING TREATMENTS) ON THE EMERGENCE OF ANTIMICROBIAL RESISTANCE THROUGHOUT THE FOOD CHAIN

A. Alvarez-Molina[1], D. Fernández[1], C. Andrés[1], M. López[1], M. Prieto[1], A. Alvarez-Ordóñez[1]
[1]Universidad de León, Food Hygiene and Technology, León, Spain

Backgrounds

The latest report on Antimicrobial Resistance (AMR) by EFSA/ECDC shows a trend towards an increase in the detection of foodborne pathogens with multi-drug resistance (MDR) and the appearance of AMR to some antibiotics which are the last effective treatment for MDR infections.

Objectives

This study assessed the contribution of disinfection and food processing treatments to the generation of AMR. We aimed at isolating AMR mutants of *Salmonella spp, Listeria monocytogenes* and *Escherichia coli* after serial (sub)lethal treatments of Ultraviolet (UV) light, non-thermal atmospheric plasma (NTAP) and biocides (peracetic acid, benzalkonium chloride, sodium hypochlorite).

Methods

Five strains of *Salmonella* spp., three of *L. monocytogenes* and four of *E. coli* were grown overnight in BHI and exposed to repeated cycles (10 cycles) of treatment at various (sub)lethal intensities. After each cycle, survivors were recovered and grown in fresh broth to initiate a new treatment cycle, and an aliquot was stored under freezing. Frozen samples were grown overnight in BHI and spread plated on Mueller-Hinton agar plates supplemented with inhibitory concentrations (established for the wild-type strains) of ampicillin, cefotaxime, ciprofloxacin, erythromycin, gentamycin, streptomycin, tetracycline, colistin and vancomycin. Resistant variants were pheno-typically characterized in terms of their resistance against various clinically relevant antibiotics.

Conclusions

Our results confirm that disinfection practices and food processing technologies used in food industries may select for AMR microorganisms that pose a serious risk to the food chain. Whole Genome Sequencing of the most relevant strains will be carried out in the future to identify mutations responsible for the AMR phenotypes.

FEMS7-2717
Food Microbiology, omics driven molecular physiology

MONITORING TETRACYCLINE RESISTANCE IN CHEESE BY FUNCTIONAL METAGENOMICS

A.B. Florez Garcia[1], *L. Vazquez*[1], *B. Mayo*[1]
[1]*IPLA-CSIC, Departamento de Microbiología y Bioquímica, Villaviciosa-Asturias, Spain*

Backgrounds

Metagenomics techniques have been successfully applied for monitoring antibiotic resistance genes in environmental, animal, and human ecosystems. However, in spite of the food chain being claimed to be a key player in the spread of antibiotic resistances, metagenomics approaches have been scarcely used in foods.

Objectives

In this work, we report on the prevalence and evolution of tetracycline resistance (Tc^r) determinants in a blue-veined, raw milk cheese by functional metagenomics.

Methods

The same cheese batch was sampled at manufacturing (day 3) and ripening (day 60) stages. Dilutions were plated in the presence of tetracycline on non-selective and selective conditions for lactic bacteria. DNA from cultures in the counting plates was then isolated and used to construct four fosmid libraries. Clones were analysed by restriction enzyme digestion, PCR and sequencing.

Conclusions

Four different Tc^r genes were identified among 300 clones, *tet*(A), *tet*(L) *tet*(M), and *tet*(S). *tet*(A), *tet*(M), and *tet*(S) were detected in libraries at day 3 and *tet*(L) was the single gene identified in the two libraries at day 60, suggesting an evolution of the Tc^r gene types along with that of the microbial populations during ripening. Six representative clones were completely sequenced. All Tc^r genes characterized in this study resided on plasmids and/or were flanked by insertion sequences, mobilization- and conjugation-associated protein-encoding genes.

- Metagenomic techniques can be a valuable tool to evaluate the resistome of cheeses and other fermented dairy products.
- Raw-milk made cheeses should be considered as reservoirs of Tc^r genes with a high potential for horizontal transference.

FEMS7-1014
Food Microbiology, omics driven molecular physiology

ADAPTIVE RESPONSE OF LISTERIA MONOCYTOGENES BIOFILMS TO A DEHUMIDIFICATION STRESS

J. Esbelin[1], C. Chambon[2], D. Viala[2], M. Hebraud[1,2]
[1]French National Institute for Agricultural Research, Centre Auvergne-Rhône-Alpes- UMR MEDiS- Site de Theix, Saint-Genès Champanelle, France
[2]French National Institute for Agricultural Research, Centre Auvergne-Rhône-Alpes- PFEMcp- Site de Theix, Saint-Genès Champanelle, France

Backgrounds

Listeria monocytogenes is a foodborne pathogen able to adhere and form biofilms on various surfaces. Associated with a high mortality rate, it is one of the major biological concerns in food hygiene. Since few years, industries attempt to reduce the environmental impact of hygiene operations in the workshops of refrigerated food processing, through optimized use of dehumidification after cleaning disinfection treatments

Objectives

Our study was focused on the adaptive response of *L. monocytogenes* cells, growing in biofilms, to a dehumidification stress mimicking food plants conditions.

Methods

The intracellular and surfaceome subproteomes were analyzed by shotgun proteomics through three complementary extraction methodologies (enzymatic shaving, biotinylation and cell fractionation). *L. monocytogenes* EGD-e and L028 biofilms were grown on stainless steel discs at 25°C during 24h, pre-adapted to 10°C and placed in a desiccation chamber where the air Relative Humidity was stabilized to 75%. The different subproteomes were analyzed after 3h and 24h dehumidification by comparison with non-stressed biofilms.

Conclusions

Among the surface proteins identified, 21 and 29 were differentially expressed during dehumidification for EGD-e and L028, respectively. Three of them were common to both strain, an autolysin, Iap and an ABC transporter. Among intracellular proteins, 38 and 65 were differentially expressed in EGD-e and L028 respectively. Five proteins were common to the two strains. The majority of these proteins belongs to information pathway (35%) and intermediary metabolism (25%) functional categories. These results could contribute to a better comprehension of mechanisms involved in the resistance and persistence of this pathogen in food plants despite the daily hygiene procedures.

FEMS7-3204
Food Microbiology, omics driven molecular physiology

VANCOMYCIN RESISTANCE IN LACTOBACILLUS SP. IS AN EVOLUTIONARY TRAIT AND NOT DEPENDENT ON FERMENTATIVE PATTERNS

G.M. Knudsen[1], B. Stuer-Lauridsen[1], Y. Agersø[1], E.J. Boll[1], A. Wind[1]
[1]Chr. Hansen, Identification, Hørsholm, Denmark

Backgrounds

Some species of *Lactobacillus* such as *L. plantarum* are vancomycin resistant, whereas others such as *L. acidophilus* are sensitive. For food and feed cultures, the European Food Safety Authority (EFSA) set cut-off values to identify antimicrobial resistant strains as they may contain transmissible antibiotic resistance. For the heterogeneous group of *Lactobacillus* sp. the cut-off values are mainly based on fermentative status i.e. homofermentative species being sensitive and heterofermentatives being resistant. However, the homofermentative *L. mali* is vancomycin resistant.

Objectives

The aim of this study was to elucidate the vancomycin resistance pattern of the *Lactobacillus* genus.

Methods

A BlastX search of the genome of *L. mali* LBA-20315 against the ResFinder database was performed, but no hits with more than 50% identity (E value > $1.9x10^{-54}$) to vancomycin resistance genes were detected. To investigate the vancomycin resistance pattern in a broader context, the susceptibility towards vancomycin of 25 species (N= 398) was investigated. In addition to *L. mali,* vancomycin resistance was observed within the homofermentative species *salivarius* and *farciminis*. The presence of genes involved in vancomycin resistance i.e. *ldh*, *aad*, *lar* and *ddl* was investigated in 11 species and no correlation with vancomycin resistance was observed. However, phenotypic vancomycin resistance correlated with different alleles of *ddl* encoding a D-alanine-D-alanine ligase also referred to as *vanA*.

Conclusions

Thus, this study indicates that vancomycin resistance is an evolutionary trait depending of variation within the *ddl* gene causing intrinsic resistance and is independent of fermentative status. Sequencing of further species is ongoing to support these conclusions.

FEMS7-2548
Food Microbiology, omics driven molecular physiology

LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY-BASED METABOLITE PROFILING OF SALMONELLA ENTERICA BIOFILM

A. Lamas[1], P. Regal[1], B. Vazquez[1], A. Cepeda[1], C.M. Franco[1]
[1]University of Santiago de Compostela, Analytical Chemistry- Nutrition and Bromatology, Lugo, Spain

Backgrounds

Salmonella enterica is responsible for foodborne diseases worldwide. *Salmonella* strains persist in the food chain partially thanks to their ability to produce biofilm. Environmental conditions and surfaces affect in different ways the biofilm formation. There are few studies defining in detail the particular metabolome of these structures under different scenarios

Objectives

The aim of this study was stablishing the metabolite profile of the *Salmonella* biofilms cells growth under different conditions.

Methods

Two strains of *S.* Typhimurium and *S.* Enteritidis with great ability to produce biofilm were used. The strains were allowed to form biofilm under different environment conditions and the metabolites were measured using LC-HRMS profiling.

Conclusions

Metabolomics appears as a good tool to explore the biofilm formation pathways in *Salmonella*.

FEMS7-1672
Food Microbiology, omics driven molecular physiology

TRANSCRIPTIONAL AND TRANSLATIONAL CHANGEOVER DURING B. SUBTILIS SPORE GERMINATION

B.N. Swarge[1], D. Beaty[1], W. Roseboom[2], S. Brul[3], C.G. de Koster[2], L.J. de Koning[2]
[1]Swammerdam Institute for Life Sciences- University of Amsterdam,
Molecular biology and Microbial Food Safety- Mass spectrometry of Biomacromolecules, Amsterdam, The Netherlands
[2]Swammerdam Institute for Life Sciences- University of Amsterdam,
Mass spectrometry of Biomacromolecules, Amsterdam, The Netherlands
[3]Swammerdam Institute for Life Sciences- University of Amsterdam,
Molecular biology and Microbial Food Safety, Amsterdam, The Netherlands

Backgrounds

In response to nutrient limitation Gram positive organisms like *Bacillus subtilis* form dormant spores. These cellular entities are survival capsules, resistant to chemical and environmental assaults. They pose challenges to the food and medical sectors. Upon contact with germinants spores return to vegetative life through a process called 'germination and outgrowth'. Spores reactivate their metabolism and develop in vegetative cells. The molecular machinery that triggers and progresses the germination is still unsettled.

Objectives

Gain insight in the germination molecular machinery by mapping the progression of the transcriptome and proteome changeover during germination and outgrowth of spores.

Methods

For the time-resolved monitoring of the transcriptome and the proteome *of B. subtilis spores* during germination, the spores are sampled in time-intervals from germination initiation to the stage of vegetative cell outgrowth. Intact and purified RNA is isolated from spores, at each time-point, for transcriptome analysis. We thus trace the different functional groups of genes expressed during the germination and outgrowth process. Next, the changes in the proteome are quantitatively monitored relative to the proteomes of ^{15}N metabolically labelled dormant spores and ^{15}N metabolically labelled vegetative cells.

Conclusions

The time-resolved transcriptomics and proteomics data provide new insight in the cellular control level of novel protein synthesis during the awakening of spores. The data will offer input for the development of a mathematical model of the germination process.

FEMS7-0408
Host-microbe interactions, gut microbiome and health

THE PHYLOGENETIC CORE OF THE HUMAN PAN-MICROBIOME

D. Aguirre de Cárcer[1]
[1]*Unidad de Genética, Ciencias, Madrid, Spain*

Backgrounds

Whether or not there is a 'core' gut microbiome, consisting of bacterial groups common to all healthy humans, remains an open question with important scientific and medical implications. Unfortunately, the strong variability in gut microbiome composition persistently observed across individuals has so far hindered efforts towards its detection.

Objectives

The present study analyzes the human gut core pan-microbiome in terms of 16S rRNA OTUs present in all individuals, where such OTUs have been produced dynamically over a range of phylogenetic depths, as opposed to using an arbitrary fixed threshold which represents the dominant practice in the field.

Methods

This approach was applied in the meta-analysis of three large and comprehensive datasets (totaling 2,243 individuals; > 150M sequences).

Conclusions

The results show that the human gut pan-microbiome contains a preeminent compositional phylogenetic core, defined in terms of discrete units of varying depth along the bacterial phylogeny, whose members are present in all individuals studied.

This result provides a new conceptual framework with great potential for advancing our understanding of the ecosystem. In addition to providing a novel perspective on (i.e.) the study of community assembly, the results obtained in this study should guide the selection of more meaningful combinations of bacterial species (or genomes) in many frequent *in vivo*, *in vitro*, or *in silico* experimental scenarios. Furthermore, the results should be used as a revised list of "most wanted" bacteria to guide future genome sequencing and isolation efforts, significantly as it also includes information on the biologically meaningful breadth of the targeted group's pan-genome.

FEMS7-1762
Host-microbe interactions, gut microbiome and health

DIFFERENCES IN THE NEONATE MICROBIOME AFTER HOME OR HOSPITAL DELIVERY

J. Combellick[1], S. Hakdong[2], S. Dongjae[3], H. Hagan[1], M.G. Dominguez Bello[4]
[1]New York University, Rory Meyers College of Nursing, New York, USA
[2]Sejong University- South Korea,
College of Life Science- Department of Food Science and Biotechnology, Seoul, Republic of Korea
[3]Sejong University, College of Life Science- Department of Food Science and Biotechnology, Seoul, Republic of Korea
[4]New York University, School of Medicine, New York, USA

Backgrounds

Hospitalization for delivery is considered a foundation of obstetric care. In the U.S. more than 99% of deliveries occur in the hospital. However in many countries—notably those in Scandinavia—home birth is recognized as a safe alternative for low-risk women. Currently there is limited investigation of how the neonatal microbiome develops in the absence of all interventions, including hospitalization. We hypothesize that hospital exposure at birth affects the neonate intestinal microbiota.

Objectives

To characterize the intestinal microbiota of newborns following home and hospital delivery and to investigate differences related to hospitalization during the first month of life.

Methods

A prospective cohort study was conducted to compare breast-fed vaginally delivered infants born at home (n=10) or in the hospital (n=10). Consecutive sampling was performed on infant feces at 7 time points from delivery through day 28. The V4 region of the 16S rRNA gene of 212 samples was sequenced using Illumina MiSeq platform. Sequences were analyzed using the QIIME pipeline.

Conclusions

Hospitalization for birth alters the microbiome of neonates. The gut microbiome differed significantly between home and hospital born neonates (PERMANOVA p=<0.005), with babies born at home showing higher fecal *Bacteroides*, and *Bifidobacterium*, *Streptococcus*, and *Lactobacillus*, at one or more time points after the first week of birth, and lower proportions of *Clostridium* (Day 21), Enterobacteriaceae family (Day 28). More research is needed to determine the health significance of these differences. These results are relevant to health care system policies that support home birth in low-risk women.

FEMS7-1320
Host-microbe interactions, gut microbiome and health

EVALUATION OF METAGENOMIC METHODS FOR THE STUDY OF THE GUT MYCOBIOME

A. Frau[1], B. Campbell[1], A. Darby[2], J. Kenny[3], N. Hall[4], J. Anson[5], C. Probert[1]
[1]University of Liverpool, Institute of Translational Medicine- Cellular and Molecular Physiology, Liverpool, United Kingdom
[2]University of Liverpool, Institute of Integrative Biology- Functional and Comparative Genomics, Liverpool, United Kingdom
[3]University of Liverpool, Institute of Integrative Biology- Centre for Genomic Research, Liverpool, United Kingdom
[4]Earlham Institute, -, Norwich, United Kingdom
[5]Royal Liverpool University Hospital NHS, Infection and Immunity, Liverpool, United Kingdom

Backgrounds

Metagenomics has revolutionized the study of the gut microbiome but most methods are tailored for bacteria.

Objectives

The mycobiome likely plays a key role in intestinal diseases and hence metagenomic methods need to be adapted to study this. Here, we evaluated two DNA extraction methods and 5 primer sets.

Methods

Genomic DNA was extracted from seven characterised fungal cultures (*C. albicans*, *C. tropicalis*, *C. neoformans*, *M. furfur*, *S. cerevisiae*, *A. fumigatus* and *P. crysogenum*) using either the PSP Spin Stool DNA kit (Stratec), with and without extra bead-beating (6 min, 0.1 and 0.5 mm zirconia beads), or the QIAamp Fast DNA Stool Mini Kit (QIAGEN), and compared using 18S rRNA qPCR. Subsequently, stool was spiked with cells and spores from the same species and gDNA extracted (as above). DNA from spiked stool samples and a mock community were amplified and sequenced. Five primer sets were tested (18S rRNA, ITS1, ITS2-2X and 28S rRNA) for HiSeq Illumina sequencing.

Conclusions

Results: 1) the extra bead-beating did not increase the extraction; 2) the Stratec Kit extracted more gDNA from *C. albicans* VS *A. fumigatus* (Cp=29.1 SD=0.52 and Cp=31.9 SD=0.51), the reverse occurred with QIAGEN kit (Cp=34.3 SD=0.74 and Cp=26.6 SD=0.06); 3) on stool DNA similar amounts of 18S rRNA copies/ng of DNA were observed with both kits. Sequencing data are being currently evaluated: this will show how extraction methods and primer sets influence analysis of the mycobiome. In conclusion, the method of DNA extraction and primer design influence results and interpretations of the mycobiome.

FEMS7-3265
Host-microbe interactions, gut microbiome and health

HOST-MICROBIOTA INTERACTIONS IN INFLAMMATORY ARTHRITIS

L. Lam[1], D. Regan-Komito[1], J. Faith[2], T. Griseri[1], F. Powrie[1]
[1]University of Oxford, Kennedy Institute of Rheumatology, Oxford, United Kingdom
[2]Mount Sinai School of Medicine, Genetics and Genomic Sciences, New York, USA

Backgrounds

The gut is the primary site of host-commensal symbiosis, but disequilibrium can drive inflammatory disease and damaging systemic immune responses. Spondyloarthropathies are a form of inflammatory arthritis, the most common of which is ankylosing spondylitis (AS), characterized by joint deformation and fusion throughout the body. Intriguingly, over 70% of AS patients have subclinical intestinal inflammation and 15-25% develop inflammatory bowel disease. Additionally, arthritis is the most common extraintestinal manifestation of IBD. Together, these findings suggest a gut-joint connection in AS pathogenesis.

Objectives

While microbial dysbiosis is strongly associated with inflammatory arthritis, it is unclear how altered intestinal community composition modulates systemic immune responses. IgA secreted at mucosal surfaces coats different species of intestinal bacteria and is a critical mediator of homeostasis. While commensal microbiota may be coated with IgA, pathogens elicit high-affinity responses, making IgA coating a powerful metric for identifying specific bacteria that trigger inflammation.

Methods

The proposed research will use differential immunoglobulin A coating of fecal bacteria coupled with 16S rRNA sequencing (IgA-SEQ) to identify immunopathogenic members of the microbiota. IgA+ and IgA- bacteria from will be collected by fluorescence-activated cell sorting in both ankylosing spondylitis (AS) patients and healthy controls.

Conclusions

In parallel with IgA-seq, patient libraries of bacterial isolates will be generated using high-throughput cultivation platforms. To identify immunomodulatory microbes, IgA+ and IgA- bacterial consortia will be screened in gnotobiotic mouse models of AS. This work has the potential to identify host-microbiota interactions that drive local intestinal inflammation and systemic joint disease.

FEMS7-2243
Host-microbe interactions, gut microbiome and health

MICROBIAL METAGENOMIC AND METATAXONOMIC STUDY OF BILIARY SAMPLES IN PATIENTS WITH CHOLEOLITHIASIS AND A CONTROL GROUP

N. Molinero[1], I. Gutierrez-Diaz[2], C. Milani[3], M. Mangifesta[3], C.M. García-Bernardo[4], A. Cabrera[5], J.I. Rodríguez[5], S. Gonzalez[6], M. Ventura[3], A. Margolles[1], S. Delgado[1]
[1]IPLA-CSIC, Microbiology and Biochemistry of Dairy Products, Villaviciosa, Spain
[2]University of Oviedo, Functional Biotogy, Oviedo, Spain
[3]University of Parma, Life Sciences, Parma, Italy
[4]HUCA, Cirugía General, Oviedo, Spain
[5]Hospital de Cabueñes, Servicio de Cirugía General y del Aparato Digestivo, Gijón, Spain
[6]University of Oviedo, Functional Biology, Oviedo, Spain

Backgrounds

In recent years, the relationship between cholesterol, bile salt metabolism, intestinal microbiota and its potential implication in health is been elucidated. However, the composition and role of the biliary microbiota remains obscure. The characterization of the human bile microbiota as a whole has been hampered by difficulties in accessing biological samples and the lack of adequate methodologies to assess molecular studies.

Objectives

The aim of this work was to generate new knowledge of human bile microbial profiles, functions and activities in patients with choleolithiasis vs healthy individuals, and identify possible dysbiosis associated with this pathology.

Methods

To characterize the biliary microbiome of patients with cholelithiasis and healthy subjects we collected bile samples from 15 gallstone patients and a comparable control group. We performed with Illumina technology high throughput sequencing of 16S rDNA amplicons, together with whole metagenome shotgun of selected bile samples.

Conclusions

A high diversity was present in the biliary microecosystem, with main fila represented: *Firmicutes*, *Bacteriodetes*, *Actinobacteria* y *Proteobacteria*. Significant differences in the relative abundance of different taxa present in the bile of both groups were found. Sequences belonging to the family *Propionibacteriaceae* were more abundant in bile samples from healthy subjects, meanwhile in patients with choleolithiasis members of the families *Bacteroidaceae, Prevotellaceae* y *Veillonellaceae* were more frequently detected.

Sequencing of total DNA from bile of three healthy subjects and functional analysis allowed us to observe that the main COG functions and categories were similar to that describe in the human intestinal microbiome.

FEMS7-3121
Host-microbe interactions, gut microbiome and health

GUT PHAGEOMICS: YET ANOTHER VIEW OF HUMAN GUT MICROBIOME

A. Shkoporov[1], A. Forde[1], K. Daly[1], S. McDonnell[1], J. Nolan[1], O. O'Regan[1], L. Draper[1], S. Stockdale[1], M. Dalmasso[1], F. Ryan[1], A. McCann[1], P. Ross[1], C. Hill[1]
[1]APC Microbiome Institute, Gut Phageomics Lab, Cork, Ireland

Backgrounds

A healthy human gut is populated by an enormously diverse and dense consortium of microorganisms. While the bacterial component of this consortium has received significant attention in the last decade, relatively little is known about viral (and in particular, the phage) component. While metagenomic sequencing provides a powerful tool for studying phage populations, little has been done to standardise or validate sequencing and bioinformatic analysis strategies, or to analyse the impact of potentially confounding factors such as storage or handling conditions.

Objectives

1) To develop and validate a protocol for metagenomic analysis of viral/bacteriophage populations in human faeces 2) To compare reproducibility and resolution of this protocol with the more traditionally used 16S rDNA amplicon sequencing methodology. 3) To analyse operator-to-operator reproducibility of the protocol, the impact of sample storage at various temperatures, the effect of repeated freeze/thaw cycles, and the addition of exogenous viral standards for absolute quantification of VLPs.

Methods

Faecal samples collected from healthy and patient cohorts were enriched for virus like particles. Viral/bacteriophage DNA was extracted and sequenced using the Illumina HiSeq platform.

Conclusions

The gut phageome sequencing method developed in this study provides an essential complementary tool to 16S amplicon sequencing for studying (and quantifying) organisms in the gut microbiome. The accuracy and reproducibility of results compare favourably between the methods and phageomics provides a valuable additional view of human gut microbiota structure and composition. Rapid storage and limited freeze-thaw cycling of faecal samples is recommended for optimum results.

FEMS7-1299
Host-microbe interactions, gut microbiome and health

THE CONSTRUCTION OF THE CURATED PRETERM GUT MICROBIOME DATABASE (PGMD)

K. Wong[1], P. Fleming[1], M. Millar[2], R. Hutchinson[1], M. Wilks[2], F. Stacey[3], K. Costeloe[1], W. Wade[4]
[1]Blizard Institute Queen Mary University of London, Centre for Genomics and Child Health, London, United Kingdom
[2]Barts and the London NHS Trust, Department of Infection, London, United Kingdom
[3]Homerton University Hospital NHS Foundation Trust, Neonatal Intensive Care Unit, London, United Kingdom
[4]Blizard Institute Queen Mary University of London, Centre for Immunobiology, London, United Kingdom

Backgrounds

The global survival rate for very low birthweight preterm babies has significantly increased over the past 30 years. Morbidity and mortality have shifted from early respiratory failure to intermediate complications such as late-onset sepsis and necrotising enterocolitis (NEC). Investigations into the pathophysiology underpinning NEC are centred on interactions between the preterm infant gut and its microbiome, with recent studies typically using culture-independent techniques such as 16S rRNA gene community profiling and next-generation sequencing to characterise the preterm gut microbiome.

Objectives

Currently available DNA sequence nucleotide databases are typically very large and often include a significant proportion of erroneous or poor-quality 16S rRNA gene sequences. The aim of this study was to construct a 16S rRNA gene Preterm Gut Microbiome Database (PGMD) that is both curated and site-specific, thus providing a robust resource for future preterm gut microbiome studies.

Methods

High-quality full-length 16S rRNA gene sequences representing all bacterial species reported in the literature for the preterm infant gut were compiled. Data from an experimental community profile dataset was used to interrogate the database. Where sequences showed ≥98.5% similarity to named species not in the database, those species were added. Un-named phylotypes represented by a high-quality reference sequence were also added. All species-level taxa were assigned a unique PGMD number.

Conclusions

The first iteration of the PGMD is comprised of 623 species-level taxa from the phyla *Acidobacteria*, *Actinobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Fusobacteria*, *Proteobacteria* and *Tenericutes*. The PGMD is a source of phylogeny-designated taxa useful for investigators involved in understanding the human microbiome.

FEMS7-0594
Microbial Ecology

TOP-DOWN REGULATION OF PROKARYOTIC GROWTH EFFICIENCY IN TEMPERATE FRESHWATER PELAGIC ENVIRONMENTS

A.S. Pradeep Ram[1], *J. Colombet*[1], *A. Thouvenot*[2], *T. Sime Ngando*[1]
[1]*Université Clermont Auvergne, LMGE- UMR CNRS 6023, AUBIERE, France*
[2]*Université Clermont Auvergne, Athos Environnement, AUBIERE, France*

Backgrounds

In aquatic systems, limited data exists on the impact of mortality forces such as viral lysis and flagellate grazing when seeking to explain factors regulating prokaryotic metabolism.

Objectives

We explored the relative influence of top-down factors (viral lysis and heterotrophic nanoflagellate grazing) on prokaryotic mortality and their subsequent impact on their community metabolism in the euphotic zone of 21 temperate freshwater lakes located in the French Massif Central.

Methods

Viral lysis was determined from frequency of visibly infected prokaryotic cells by transmission electron microscopy whereas heterotrophic nanoflagellate grazing potential was calculated from flagellate clearance rate estimates.

Conclusions

Prokaryotic growth efficiency (PGE, index of prokaryotic community metabolism) determined from prokaryotic production and respiration measurements varied from 5 to 74% across the lakes. Viral and potential grazer induced mortality of prokaryotes had contrasting impact on PGE. Potential flagellate grazing was found to enhance PGE whereas viral lysis had antagonistic impacts on PGE. The average PGE value in the grazing and viral lysis dominated lake water samples was 35.4% (± 15.2%) and 17.2% (± 8.1%) respectively. Selective viral lysis or flagellate grazing on prokaryotes together with the nature of contrasted substrates released through mortality processes can perhaps explain for the observed variation and differences in PGE among the studied lakes. The influences of such specific top-down processes on PGE can have strong implications on the carbon and nutrient fluxes in freshwater pelagic environments.

FEMS7-2199
Microbial Ecology

COMMUNITY ECOLOGY OF AEROBIC ANOXYGENIC PHOTOTROPHIC (AAP) BACTERIA AT VARIOUS TEMPORAL AND SPATIAL SCALES

A. Auladell[1], P. Sánchez[1], J.M. Gasol[1], I. Ferrera[1]
[1]Marine Sciences Institute - ICM-CSIC, Dept. of Marine Biology, Barcelona, Spain

Backgrounds

Aerobic anoxygenic phototrophs (AAPs) are photoheterotrophic microorganisms that require organic substrates for growth, but can derive a portion of their energy harvesting light using bacteriochlorophyll *a*. Their ecology, metabolism and physiology have been intensively studied during the last two decades, but there is still a lack of large-scale analyses needed to unravel their spatio-temporal dynamics.

Objectives

Here, we focus in the community ecology of AAPs by analyzing the diversity of their marker gene *pufM* in large metagenetic (PCR-amplification and Illumina sequencing) and metagenomic datasets from 2 global circumnavigation expeditions, Tara Oceans and Malaspina, as well as from a decade-long time-series in the NW Mediterranean Sea (Blanes Bay Microbial Observatory).

Methods

At the spatial scale, we observed that variation in community structure was driven both by geographical location and by environmental conditions. In most stations phylogroups associated with the *Gammaproteobacteria*, *Roseobacter* and *Rhodobacter* were dominant but, interestingly, in some stations half of the sequences could not be associated to any defined phylogroup. At temporal scales, seasonality clearly differentiated AAP communities and half of the variation was explained by day length, temperature, water transparency, chlorophyll *a*, salinity and phototrophic nanoflagellates abundance. Comparing spatial and temporal scales, we observed that although higher richness was observed at temporal scales in a single site, dissimilarities between samples at spatial scales presented more variation, which increased with geographical distance.

Conclusions

Our work shows that a combination of large genetic studies with global and eulerian sampling have the most powerful potential to discern the spatio-temporal dynamics of marine microbial communities.

FEMS7-1403
Microbial Ecology

MICROBIAL SUCESSION DYNAMICS IN THE FOREFIELD OF BREIÐAMERKURJOKULL GLACIER (ICELAND)

A. De los Ríos[1], D. García-Angulo[2], J. Curiel[2], J. Durán[3], R. Ortiz-Alvárez[4], A. Torralba-Burrial[5], S. Heiðmarsson[6], S. Pérez-Ortega[7]
[1]Museo Nacional de Ciencias Naturales-CSIC, Biogeochemistry and microbial ecology, Madrid, Spain
[2]Museo Nacional de Ciencias Naturales-CSIC, Biogeography and global change, Madrid, Spain
[3]Center for Functional Ecology- University of Coimbra, Live Sciences, Coimbra, Portugal
[4]Centre for Advanced Studies of Blanes CEAB-CSIC, Continental Ecology, Blanes, Spain
[5]Cluster of Energy- University of Oviedo, Environment and Climate Change, Oviedo, Spain
[6]Icelandic Institute of Natural History- IS-600, Botany, Akureyri, Iceland
[7]Real Jardín Botánico-CSIC, Mycology, Madrid, Spain

Backgrounds

One key consequence of glacier recession, as effect of climatic change, is the creation of new habitats for colonization. In glacier forefields, primary succession occurs simultaneously in soils and rocks recently discovered offering a type of natural experiment in which temporal colonization dynamics can be analyzed.

Objectives

A chronosequence established at Breiðamerkurjökull Glacier forefield, was used as a framework to analyze primary microbial succession processes in subarctic regions. This outlet glacier stretches to southeast from Vatnajökull Glacier and has been dramatically retreating during the 20th century.

Methods

Soil samples from different succession stages were collected. Microbial community structure was analyzed by high-throughput amplicon sequencing. Potential microbial activity (microbial respiration, N mineralization) as well as different soil attributes were also measured in these samples.

Conclusions

Microorganisms play a fundamental role in the initial colonization of exposed soils after glacier ice retreat. They are the only colonizers at soils close to the glacier front and are functionally relevant in later successional stages. High-throughput amplicon sequencing of fungal and bacterial communities revealed that the structure of microbial communities from soils close to the glacier front considerably differed to that found in later successional stages. After a first abrupt change in community composition at the beginning of the succession, changes occur smoothly but showed a clear trend along the chronosequence. We found a significant decrease in soil pH, and a significant increase in the size of both, the soil organic matter and the organic and mineral N pools, in parallel with the succession process. Rates of microbial respiration and N mineralization also significantly increased with time of exposure after glacier retreat. Hence, our results demonstrate that primary succession along this chronosequence is accompanied by both a replacement of microbial taxa and changes in soil functionality.

FEMS7-1011
Microbial Ecology

SOIL MICROBIAL DIVERSITY: A COMPARISON OF METAGENOMICS AND CULTUROMICS APPROACHES

C. Dumolin[1], *P. Vandamme*[1]
[1]*Ghent University, Biochemistry and Microbiology, Gent, Belgium*

Backgrounds

Novel insights in the tree of life revealed staggering numbers of uncultivated bacterial lineages. Harnessing this novel biotechnological potential requires prior cultivation. Although metagenomics studies are considered the gold standard for analyzing microbial diversity, large scale isolation and identification campaigns, i.e. ”culturomics “, may reveal organisms that went undetected via metagenomics. A combined approach, is therefore paramount to determine the microbial diversity and to exploit its biological functionalities.

Objectives

The aim of the present study was to determine the microbial diversity in an old-growth forest soil sample using both metagenomics and culturomics.

Methods

A plain soil sample of the Aelmoeseneie forest (Gontrode, Belgium) was collected. For metagenomic analyses, DNA was extracted using the Mobio Powersoil kit. Total DNA was sequenced on the Illumina HiSeq platform. The cultivable microbial diversity of the same soil sample is studied via an in-house developed culturomics pipeline, based on the automated picking of isolates and dereplication via MALDI-TOF MS. We aim to isolate and identify 25,000 bacterial isolates.

Conclusions

Shotgun metagenomic sequencing yielded >237,000 reads, correlating to a bacterial richness of >800 species. This diversity is dominated by *Proteobacteria* (55%), *Actinobacteria* (35%) and *Acidobacteria* (3%). *Proteobacteria* are mainly represented by *Alphaproteobacteria* with *Rhizobiales* (78%) and *Rhodospirillales* (7%) as main orders. *Actinobacteria* are dominated by the *Corynebacteriales* order (31%); interestingly, 8% of the total DNA sampled represented *Mycobacterium* reads. The culturomics analysis is ongoing; in summer 2017 half of the culturomics data, i.e. referring to the isolation and identification of about 12,500 isolates, will be available and presented.

FEMS7-2295
Microbial Ecology

METAGENOMIC SNAPSHOT: MEASURING PATTERNS BY GEOGRAPHICAL LOCATIONS IN MARINE METAGENOME DATA USING NEWLY ADOPTED GENOTYPING-BY-SEQUENCING

H.J. Seong[1], C.Y. Hwang[2], H.H. Won[3], W.J. Sul[1]
[1]Chung-ang University, Systems Biotechnology, Anseong, Republic of Korea
[2]Korea Polar Research Institute, Division of Polar Biology and Ocean Sciences, Incheon, Republic of Korea
[3]Sungkyunkwan University- Samsung Medical Center,
Samsung Advanced Institute for Health Sciences and Technology, Seoul, Republic of Korea

Backgrounds

Dealing with huge quantity and high complexity of metagenomic data is a very difficult and time-consuming process. In addition, a new metagenomic approach is required to simply identify genetic variation.

Objectives

We demonstrated more effective metagenomic approach to analyze particular sequences related to restriction enzyme sites for targeting reduced numbers of genes than to estimate whole genome sequences.This approach was applied to analyze the marine metagenome by genotyping-by-sequencing (GBS) method, which was used to analyze plant genomes with large genome sizes.

Methods

Metagenomic GBS was demonstrated with marine epipelagic samples collected on 10 locations across from East Sea to Bering approximately 8000 km apart on July, 2013. These samples were processed by a restriction enzyme *Ape*KI and then sequenced by Illumina HiSeq, yielding 74.4 million reads. These restriction site-associated sequences were analyzed to characterize taxonomic diversity and functional profiling of microbial community directly. Moreover, metagenomic GBS reads were grouped, aligned, and Shannon entropy-analysis using UCLUST, MUSCLE, and in-house script from Oligotyping respectively. Entropy in sequence variation among sorted clusters by functional category was measured to evaluate their rate of SNPs variations.

Conclusions

Nucleotide and amino acid substitutions were discovered that indicate micro genetic diversity associated with evolutionary histories according to geographical patterns. Besides profiling microbial community and functional pathway, metagenomic GBS can analyze evolutional study based on nucleotide variation. GBS to metagenomic analysis was applied as a snapshot for understanding the highly dynamic ecosystem quickly to resolve budget and time-consuming issues with identification of micro genetic diversity through comparing each samples.

FEMS7-0773
Microbial Ecology

ESTIMATING MICROBIAL COMMUNITY STRUCTURE USING METABOLIC NETWORKS

K. Takemoto[1]
[1]*Kyushu Institute of Technology, Department of Bioscience and Bioinformatics, Iizuka, Japan*

Backgrounds

Microbes interact with one another via metabolic interactions etc., and they compose ecological communities. It is important to untangle such ecosystems in medical and environmental sciences. In this context, microbial ecological networks are often studied. In general, such networks are estimated based on time-series data on species abundance, obtained from 16S rRNA data, for example. However, such estimation methods have fiscal constraints; moreover, they are not useful for understating microbial ecosystems at the molecular level, despite the importance of metabolite interactions in microbial ecosystems.

Objectives

Thus, I developed a novel method for estimating microbial community structure using metabolic network analysis: Estimator of COmmunity Structure based on MetabOlic networkS (ECOSMOS).

Methods

For this, I considered reverse ecology [R. Levy, E. Borenstein, Adv. Exp. Med. Biol. 751, 329 (2012)]. In particular, cooperative interactions (i.e., complementary support of nutrients) and competitive interactions (i.e., scramble for nutrients) are calculated based on nutrient metabolites identified using graph-theoretic algorithms. In addition to this, I used random matrix theory [S. Suweis et al. Nat. Commun. 6, 10179 (2015)], and evaluated ecological community stability based on the strength of interactions and species abundance.

Conclusions

ECOSMOS considers metabolic interactions between organisms, and it estimates ecological networks from at least one sample. I applied ECOSMOS to actual examples, and found interesting results. For example, the gut microbiota in healthy subjects were highly stable. Cooperation and stability of soil microbial community increase after environmental perturbations. ECOSMOS enhances our understanding of microbial ecological community. ECOSMOS is available from takemoto08.bio.kyutech.ac.jp/ecosmos-lite/

FEMS7-0576
Molecular Taxonomy, Genomics and Phylogeny

REAFFILIATION OF EDWARDSIELLA TARDA FISH ISOLATES TO THE NEW SPECIES EDWARDSIELLA PISCICIDA AND EDWARDSIELLA ANGUILLARUM

N. Buján[1], S. Balboa[1], J. L. Romalde[1], A. E. Estévez[1], B. Magariños[1]
[1]Fac. Bioloxía-CIBUS & Instituto de Acuicultura,
Microbioloxía e Parasitoloxía- Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Backgrounds

Edwardsiella is a genus belonging to the family *Enterobacteriaceae*, described in 1965 by Ewing and MacWhorter. Until 2012, the genus consisted of 3 species: *E. hoshinae*, *E. ictaluri* and *E. tarda*. Based on genetic studies two novel species, *E. piscicida* and *E. anguillarum* were described in 2013 and 2015 respectively. *E. piscicida* compiled only pathogenic strains isolated from fish showing phenotypic characteristics identical to *E. tarda*. The capacity to ferment mannitol and arabinose is the unique characteristic distinguishable for *E. anguillarum*, but is not enough to a *bona fide* identification

Objectives

The description of the new *Edwardsiella* species made necessary a deeper study of the phylogeny of the genus to clarify the position of isolates previously classified as *E. tarda*.

Methods

A total of 57 strains and 4 genomes retrieved from the GenBank database of *Edwarsiella* were used in this work. All strains were previously identified by classical phenotypical tests.
Seven gene loci were selected, including 16S rRNA gene, *adk*, *atp*D, *dna*J, *gln*A, *hsp*60 and *tuf*, to perform the MultiLocus Sequence Analysis (MLSA).
The OrthoANI values among the genomes were calculated using OAT (v0.93). Moreover, estimated DNA-DNA hybridization was determined by the genome-to-genome distance calculator (GGDC2.1).

Conclusions

The results indicate that *Edwardsiella* isolates obtained from fish and previously identified as *E. tarda* should be reclassified as *E. piscicida* or *E. anguillarum*. The scarce phenotypic differences among *E. tarda*, *E. piscicida* and *E. anguillarum* made necessary the use of molecular techniques to a correct identification of these species.

FEMS7-0608
Molecular Taxonomy, Genomics and Phylogeny

COMPARATIVE GENOMIC FEATURES OF BACILLUS VELEZENSIS, BACILLUS AMYLOLIQUEFACIENS AND BACILLUS SIAMENSIS

B.H. Chun[1], K.H. Kim[1], S.E. Jeon[1], C.O. Jeon[1]
[1]Chung-Ang University, Department of Life Science, Seoul, Republic of Korea

Backgrounds

Three *Bacillus* groups (*B*. *velezensis*, *B*. *amyloliquefaciens*, and *B*. *siamensis*) are not differentiated based on 16S rRNA gene sequences because they are very closely related each other. Therefore, using pan-genomes of the three *Bacillus* groups we investigated the phylogenetic relatedness and compared their genomic and metabolic features.

Objectives

The aim of this study is to investigate the comparative genomic and metabolic features of three *Bacillus* groups with high phylogenetic relatedness.

Methods

Genomes that were possibly affiliated to the three *Bacillus* groups were retrieved from GenBank based on >98% of 16S rRNA gene sequence similarities and >95% of average nucleotide identity values to their respective type strains. After removing low quality genomes using the CheckM program, the comparative genomic and metabolic features of three *Bacillus* groups were investigated using pan-genome.

Conclusions

The 64 high quality genomes of *B*. *velezensis* (54), *B*. *amyloliquefaciens* (6), and *B*. *siamensis* (4) were selected and A phylogenetic analysis based on 16S rRNA gene sequences showed that the three *Bacillus* groups were undifferentiated phylogenetically, while a phylogenetic analysis using 1,957 core genes showed that they were clearly differentiated into three different phylogenetic lineages. The three *Bacillus* groups had similar COG distribution patterns, but their molecular phenotype-based relatedness was differentiated into three groups. The comparative genome analysis of three *Bacillus* groups showed that all *B*. *velezensis* strains harbor a macrolactin gene cluster, while all *B*. *siamensis* strains have a xanthine metabolic gene cluster.

FEMS7-0484
Molecular Taxonomy, Genomics and Phylogeny

ENTOMOPATHOGEN ID: A MULTI-LOCUS SEQUENCE ALIGNMENT RESOURCE FOR ENTOMOPATHOGENIC FUNGI

C. Dunlap[1], J. Ramirez[1], D. Labeda[2]
[1]NCAUR, Crop Bioprotection, Peoria, USA
[2]NCAUR, Mycotoxin Prevention and Applied Microbiology research unit, Peoria, USA

Backgrounds

The ability to correctly identify entomopathogenic fungi is an important step in developing biopesticides and effectively communicating research results. Over the years, identifying entomopathogenic fungi has evolved from a system based on diagnostic morphological and physiological characters to molecular DNA based systems. Internal transcribed spacer (ITS) sequences have become the barcode of choice when identifying unknown fungi. While ITS sequencing is a great tool for identifying unknown fungi, it often lacks sufficient resolution to accurately identify common entomopathogenic fungi. The problem is further compounded by not having a curated database of reference sequences based on type material to compare sequences against.

Objectives

Develop a web-based Multi-Locus Sequence Alignment resource for common Hypocrealean entomopathogenic fungi.

Methods

The Entomopathogen ID Multi-Locus Sequence Alignment resource is a curated sequence database built on sequences from MLSA and other taxonomic studies of common Hypocrealean entomopathogenic fungi spanning the taxonomic families of Cordycipitaceae, Clavicipitaceae and Ophiocordycipitaceae.

Conclusions

The database provides a new resource to improve the taxonomic characterization of this group of fungi.

FEMS7-0600
Molecular Taxonomy, Genomics and Phylogeny

POLYSACCHARIDE DECOMPOSERS AMONG HALOPHILIC ARCHAEA - A SYSTEMATIC GENOMIC AND PHENOTYPIC APPROACH

R.L. Hahnke[1], J.P. Meier-Kolthoff[1], M. Göker[1], B.J. Tindall[1]
[1]DSMZ, Microorganisms, Braunschweig, Germany

Backgrounds

Some *Bacteria* and *Archaea* taxa are known to thrive in carbohydrate-rich habitats as decomposers of particulate organic matter. In contrast, few polysaccharides have been tested and few taxa have been systematically investigated for polysaccharide degradation.

Objectives

With the aim of obtaining deeper insights into the evolution of carbohydrate metabolism in *Halobacteria*, we tested type strains in the family *Natrialbaceae* (*Archaea*) for their potential and ability to decompose polysaccharides.

Methods

Strains were incubated in media with NaCl concentrations of 10–32%, low nutrient concentrations and azurin-crosslinked polysaccharides in microtiter plates. Genome-scale phylogenies were inferred from whole proteomes using the Genome BLAST Distance Phylogeny (GBDP) approach. Genes encoding carbohydrate active enzymes were retrieved from the genome by matching the CAZy database.

Conclusions

Strains belonging to the genera *Halopiger* and *Halostagnicola* were rich in CAZymes, but strains belonging to the genera *Natronococcus*, *Natrinema*, *Natronorubrum*, *Natronolimnobius*, *Natronobacterium*, *Halobiforma* and *Halovivax* were not. Surprisingly, the diversity of polysaccharide decomposition differed significantly within the genera *Haloterrigena* and *Natrialba*. Galactomannan, pachyman, Barley-β-glucan and CM-cellulose were hydrolyzed only above 23% NaCl. Furthermore, the ability to grow on polysaccharides is directly correlated to the presence of genes of the semi-phosphorylated Entner-Doudoroff pathway in their genomes.

The results presented here demonstrate the advantage of a combined genomic and phenotypic approach to the systematic high-throughput screening of polysaccharide decomposition in prokaryotic taxonomy. Furthermore, the results provide additional information for the description and classification of microbial species and led to the discovery of so far hidden physiological features.

FEMS7-1107
Molecular Taxonomy, Genomics and Phylogeny

A PROTEIN SEQUENCE SET FOR MULTILOCUS SEQUENCE ANALYSIS (MLSA) AND PHYLOGENOMIC AFFILIATION IN THE MARINE ROSEOBACTER LINEAGE

B. Nogales[1], *M. Mas-Lladó*[2], *R. Bosch*[1]
[1]*University of the Balearic Islands UIB and IMEDEA UIB-CSIC, Microbiology, Palma de Mallorca, Spain*
[2]*University of the Balearic Islands, Microbiology, Palma de Mallorca, Spain*

Backgrounds

The Roseobacter lineage (*Rhodobacteraceae*, *Alphaproteobacteria*) is an important group of marine bacteria. Robust phylogenetic reconstructions using concatenates of a variable number (i.e. 52-78) of single-copy, conserved protein sequences have been done. This provides a backbone for the affiliation of new isolates. However, the pace of genome sequencing in this lineage requires continuous reevaluation of a *core* protein set useful for phylogeny.

Objectives

In this study we propose a set of fourteen protein sequences for MLSA in the Roseobacter lineage that reproduces correctly the phylogeny of the lineage and can be used for the affiliation of new isolates.

Methods

To define the MLSA protein set we calculated a *core* proteome using 96 Roseobacter genome sequences (114 proteins). We retrieved the conserved residues, concatenated them and calculated a phylogenetic tree by maximum parsimony. After calculating individual trees for each protein we selected fourteen of them (4,462 amino acid positions) whose concatenated sequences reproduced better the topology of the *core* proteome tree. To check the usefulness of this MLSA protein set we included 69 additional genome sequences and calculated a new tree (4,412 aligned positions).

Conclusions

We observed differences only in the grouping of two isolates belonging to the two most deeply-branching genomic groups and in the intra-group branching order of a third genomic group. Therefore, most of the isolates were placed in the expected genomic group and genus, confirming the usefulness of this approach for the rapid and robust affiliation of new isolates of the Roseobacter lineage.

FEMS7-0679
Molecular Taxonomy, Genomics and Phylogeny

ARCOBACTER LACUS SP. NOV. AND ARCOBACTER CAENI SP. NOV., TWO NEW SPECIES ISOLATED FROM RECLAIMED WATER

A. Pérez-Cataluña[1], M.J. Figueras[1], N. Salas-Massó[1]
[1]Universitat Rovira i Virgili- IISPV,
Unidad de Microbiología - Departamento de Ciencias Médicas Básicas - Facultad de Medicina y Cien cias de la Salud, Reus, Spain

Backgrounds

The genus *Arcobacter* belongs to the family *Campylobacteraceae* and differs from *Campylobacter* on the basis of the capacity of the species to growth at low temperatures. Since its description in 1991, 24 species had been added to the genus.

Objectives

In the present study we performed a taxonomic study using a polyphasic approach including genome information of two strains (RW43-9 and RW17-10) recovered from secondary treated wastewater.

Methods

The first identification with the *rpoB* gene sequencing, revealed on the basis of the phylogenetic analysis that those strains formed two potentially new linages. A phylogenetic analysis of the 16S rRNA gene and of the concatenated sequences of 5 housekeeping genes (*atpA*, *gyrA*, *gyrB*, *hsp60* and *rpoB*) i.e. MLPA was performed. In addition, genomic DNA was sequenced and used for genomic comparison. The Average Nucleotide Identity (ANI) and the *in silico* DNA-DNA hybridization (*is*DDH) was calculated between the genomes of the two new taxa and their nearest species. Phenotypic characterization of these two strains was also performed.

Conclusions

The differential phenotypic characteristic between the strains RW 43-9 and RW17-10 and its nearest species are the ability to growth in TSA supplemented with 5% sheep blood at 37ºC and 42ºC in anaerobiosis and the inability to growth in minimal medium, respectively. These findings together the MLPA analysis and the values of ANI (<96%) and isDDH (<75%), demonstrate that these strains represent two new species, for which the names *Arcobacter lacus* (type strain RW43-9^T=CECT 8994^T=LMG 29062^T) and *Arcobacter caeni* (type strain RW17-10^T =CECT 9140^T=LMG 29151^T) are proposed.

FEMS7-0414
One Health and human Pathogenicity

IGG PROTEASES IN CANINE MYCOPLASMAS

D.R. Brown[1], *D.L. Michaels*[1]
[1]*University of Florida, Infectious Diseases and Pathology, Gainesville Florida, USA*

Backgrounds

Certain streptococci encode endoproteases that cleave IgG at a specific epitope between the hinge and CH2 glycosylation sites. The IgG consequently loses all lymphocyte FcγR-mediated effector function and cannot activate complement. Orthologs differ among streptococcal species in their narrow selectivity for IgG of the hosts to which the bacteria are normally restricted, a feature thought to reflect fine-tuned adaptation to host immune defenses.

Objectives

We sought to identify streptococcal IgG protease orthologs in the genomes of mycoplasmas.

Methods

Mycoplasma canis, *Mycoplasma cynos*, *Mycoplasma opalescens*, *Mycoplasma spumans* and *Ureaplasma canigenitalium*, all normally found in dogs, encode the core sequence of the enzyme (GenBank Protein Cluster PCLA_442768). Its genomic context differs among species and some have N- or C-terminal fusions with other mycoplasmal proteins, which suggests origins by horizontal transfer possibly from *Streptococcus canis*. We compared non-synonymous to synonymous amino acid substitutions within the core sequence in streptococci and mycoplasmas but found little evidence that site-specific diversifying selection drives adaptation to the IgGs of different hosts. A predicted mannose and glucosamine ligand-binding exosite, possibly responsible for inital interactions with substrate IgG, was conserved in *M. canis*, *M. opalescens* and *U. canigenitalium* but degenerate in *M. cynos* and *M. spumans*. The IgG in normal dog serum was degraded in the signature pattern of streptococcal IgG proteases when incubated with *M. canis* but not with *M. cynos*.

Conclusions

We conclude that the IgG protease in certain mycoplasmas is evolutionarily, structurally and functionally related to streptococcal orthologs and may confer previously unrecognized immunosuppressive effects during mycoplasmosis of dogs.

FEMS7-0307
One Health and human Pathogenicity

DISULFIDE BOND FORMATION IN GASTRIC PATHOGEN HELICOBACTER PYLORI: AN ACHILLAES' HEEL FOR SECRETION OF PRO-INFLAMMATORY VIRULENCE PROTEINS

C. Creuzenet[1], J. Lester[1], S. Kichler[1], H. Atanassov[2], C. Bodet[2], B. Oickle[1], S. Fairweather[1], M. Perivier[2], A. Oberc[1], J. Chahal[1], D. Ratnayake[1], C. Burucoa[2]
[1]Western University, Microbiology and Immunology, LONDON, Canada
[2]University Hospital, LITEC, Poitiers, France

Backgrounds

H. pylori causes gastritis, gastric ulcers and cancers but the mechanisms of virulence are not fully understood. It produces secreted proteins which may play a role in eliciting gastric inflammation, including the Helicobacter cysteine rich protein HcpE (HP0235) whose biological function is unknown.

Objectives

To investigate if HcpE is secreted by *H. pylori* and is involved in host / pathogen interactions, and identify components essential for its production.

Methods

This work uses anti-HcpE ELISA and Western blots, knockout mutagenesis, phenotypic analyses and biochemical assays.

Conclusions

We demonstrate that HcpE is secreted by many strains as a soluble protein and in association with outer membrane vesicles. We show that infected patients produce anti-HcpE antibodies, indicating in situ HcpE production. We show that HcpE comprises many disulfide bonds and identify DsbK (HP0231) as a folding factor necessary for HcpE production, and show that recombinant DsbK can refold unprocessed, reduced HcpE *in vitro*. This highlights the first biologically relevant substrate for DsbK. Furthermore, we show that DsbK has DiSulfide Bond (Dsb) forming activity on reduced lysozyme and has DsbA-like activity upon expression in *E. coli*, despite its similarity with DsbG. Also, we show a role of DsbK in redox homeostasis in *H. pylori*. Finally we show an important role for DsbK and HcpE in host-pathogen interactions, including murine gastric colonization and pro-inflammatory cytokine production in human gastric explants and in murine splenocytes. Both proteins will be investigated as therapeutic targets to treat *H. pylori* infections and prevent gastric ulcers and cancers.

FEMS7-0087
One Health and human Pathogenicity

PIECING TOGETHER YSCX FUNCTION: THE CRITICAL PROTEIN IN YERSINIA TYPE III SECRETION SYSTEM

J.M. Gurung[1], A.A.A. Amer[1], S. Chen[2], M.S. Francis[1]
[1]Umeå University, Department of Molecular Biology, Umeå, Sweden
[2]Chinese Academy of Sciences, Wuhan Institute of Virology, Wuhan, China

Backgrounds

Yersinia bacteria utilize a type III secretion system (T3SS) to target effector toxins into the host cells which promote mutualistic or pathogenic associations. T3SS's are comprised of ~25 structural proteins many of whose functions remain obscure. In *Yersinia*, YscX and YscY are two poorly characterised proteins. However, the absence of Ysc-Yop T3SS in *yscX* or *yscY* null mutant suggests that YscX and YscY are important Ysc-Yop T3SS constituents. This may arise from a need to form a binary interaction involving YscX-YscY or a ternary interaction with the inner membrane export component YscV.

Objectives

Study the contribution of YscX secretion towards T3S function.

Methods

To understand the role of YscX, we initially investigated the functional interchangeability between genetically conserved members of YscX-YscY protein families from diverse bacteria. Our study suggested that the function of YscX might be specific to *Yersinia* despite reciprocal binding to YscY and YscV family members. To further dissect the uniqueness of YscX function, we scrutinized the role of YscX N-terminus in secretion of itself and other T3S proteins. Site-directed mutagenesis and defined domain swapping revealed YscX N-terminus to be central for Ysc-Yop activity as it prevented YscF needle assembly and abolished T3SS activity. Critically, the inability of these YscX variants to impede T3SS was not due to the disruption of their secretion ability or destabilization of binary and ternary interactions.

Conclusions

Hence, the YscX N-terminus is dual functional; on the one hand it is a secretion recognition motif and on the other, a novel structural component necessary for the correct assembly of the Ysc-Yop T3SS.

FEMS7-1223
One Health and human Pathogenicity

PSEUDOMONAS AERUGINOSA PHOSPHOLIPASES AND LECTINS ARE POTENTIAL THERAPEUTIC TARGETS

F. Kovacic[1], F. Bleffert[1], T. Schwabroch[1], C.H. Strunk[1], M. Caliskan[1], J. Granzin[2], M. Siebers[3], P. Dörmann[3], L. Rahme[4], R. Batra-Safferling[2], K.E. Jaeger[1,5]
[1]Institute of Molecular Enzyme Technology- Heinrich-Heine-University Düsseldorf, Bacterial Enzymology, Jülich, Germany
[2]Institute of Complex Systems- Forschungszentrum Jülich GmbH, ICS-6: Structural Biochemistry, Jülich, Germany
[3]Institute of Molecular Biotechnology- Universität Bonn, IMBIO, Bonn, Germany
[4]Harvard Medical School, Department of Microbiology and Immunology, Boston, USA
[5]Institute of Bio- and Geosciences- Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany

Backgrounds

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen infecting immunocompromised humans with high mortality rates. Many virulence factors, among them phospholipases and lectins contribute to biofilm formation and high antibiotic resistance of *P. aeruginosa*.

Objectives

Phospholipases are involved in bacterial adaptation, host membrane damage and modulation of host lipid signaling. We have studied a novel phospholipase A which may contribute to the biosynthesis of lipid messengers related to virulence and biofilm formation. The lectin LecB is located at the cell surface and mediates bacterial attachment to human tissue during initial biofilm formation. We have tried to elucidate the so far unknown LecB secretion pathway.

Methods

PlbF as a novel phospholipase A of *P. aeruginosa* was shown to hydrolyze membrane phospholipids *in vitro* and *in vivo* releasing medium chain fatty acids. It acts as a virulence factor as shown by a *Drosophila melanogaster* infection model and is involved in biofilm formation. Screening of a transposon mutant library of *P. aeruginosa* using a high-throughput enzyme-linked lectin assay resulted in the identification of approximately 30 strains significantly affected in LecB secretion with targeted genes involved in the biogenesis of the flagellum and type-IV pili involved in cell attachment and biofilm formation.

Conclusions

The novel phospholipase PlbF may be linked to a virulence mechanism involving cell signaling in *P. aeruginosa*. The lectin LecB may be co-secreted with flagellin *via* a type-III secretion system. These data suggest that that lectin and phospholipase may represent potential targets for treatment of *P. aeruginosa* infections.

FEMS7-0131
One Health and human Pathogenicity

PROPIONIC ACID INDUCES RAPID EVOLUTION OF CROHN'S DISEASE ASSOCIATED AIEC

M. Ormsby[1], L. Meikle[1], A. Mcintosh[1], S. Johnson[1], R. Goldstone[2], C. Edwards[3], D. Smith[2], D. Wall[1]
[1]Infection- Immunity and Inflammation, Bacteriology, Glasgow, United Kingdom
[2]Biological Chemistry- Biophysics and Bioengineering, Heriot-Watt University, Edinburgh, United Kingdom
[3]School of Medicine, Human Nutrition, Glasgow, United Kingdom

Backgrounds

Crohn's Disease (CD) is a multi-factorial disease that occurs in genetically susceptible individuals, resulting in inflammation of the digestive tract. Concurrently, the use of antibacterial compounds, particularly short chain fatty acids (SCFAs), has increased dramatically in recent years in both agriculture and human food. One such SCFA, propionic acid (PA), inhibits *Salmonella* and *Campylobacter* colonisation of poultry. Here we show that the bacterial pathotype adherent and invasive *Escherichia coli* (AIEC), that has been isolated in increased numbers from CD patients, can use PA as a carbon source for growth.

Objectives

Our work aims to elucidate the adaptation that pre-exposure to PA may have on the ability of AIEC to colonise the human intestinal tract, outcompeting the native microflora.

Methods

Continued growth of the AIEC type strain LF82, in PA, resulted in; an increased rate of growth; greater adherence to Caco-2 intestinal epithelial cells; and a greater propensity to form biofilms under anaerobic conditions. PA use was also seen to induce shedding of inflammatory bacterial micro-compartments, with upregulation of both the *pdu* and *eut* operons, for 1,2-propanediol and ethanolamine use, respectively. Both metabolites are known to allow pathogens such as *Salmonella* Typhimurium to out-compete the native microflora during intestinal inflammation.

Conclusions

As antibiotic use has previously been implicated in the horizontal transmission of *E. coli* between poultry and humans, this work highlights the risk associated with using alternative antimicrobials such as PA in both agriculture and human food potentially evolving *E. coli* strains that are specifically adapted to life in the inflamed CD gut.

FEMS7-1064
One Health and human Pathogenicity

CHARACTERIZATION OF THE LUNG MICROBIOME IN LEGIONELLA-ASSOCIATED PNEUMONIA AND ITS EVOLUTION DURING ANTIBIOTIC TREATMENT

A.E. Pérez Cobas[1,2], C. Ginevra[3,4], C. Rusniok[1,2], J. Sophie[3,4], C. Buchrieser[1,2]
[1]Institute Pasteur, Biology of Intracellular Bacteria, Paris, France
[2]CNRS UMR 3525, 75724, Paris, France
[3]Centre National de Référence des Légionelles, Hospices Civils de Lyon, Lyon, France
[4]Centre International de Recherche en Infectiologie- Université Lyon 1- UMR CNRS 5308- U1111 Ins erm, Ecole Normale Supérieure de Lyon, Lyon, France

Backgrounds

Pneumonia represents a major public health problem with a high rate of morbidity and mortality worldwide. Despite the explosion of microbiome (MC) analyses, very little is known about the lung MC, especially with respect to bacterial pneumonia. The lung MC seems to contribute to the stimulation of the immune system and to inflammation. It thus plays possibly a role in the response to lung infections.

Objectives

Here we characterized the dynamics of the lung MC during bacterial infection and antibiotic therapy.

Methods

We collected broncheoalveolar samples from patients with pneumonia caused by *Legionella pneumophila* during around two months of hospitalisation and performed a longitudinal analysis of their lung MC. We characterized the bacterial and fungal diversity of these samples by deep sequencing (Illumina) of the 16S rRNA gene (bacteria) and the ITS region (fungi).

Conclusions

During infection the bacterial fraction of the lung MC was characterized by a low diversity and a dominance of the pathogen, as *L. pneumophila* represented more than 50% of the identified bacteria. Antibiotic treatment led to a strong change in the MC as we observed a marked decrease in the abundance of *L. pneumophila* and an increase in the bacterial diversity. The commonly identified bacteria of the lungs such as *Prevotella*, *Fusobacterium* or *Staphylococcus* were recovered. In contrast, the composition and diversity of the fungal fraction was more homogeneous. However, the fungal diversity was higher during infection probably because of a colonization advantage of fungi due to the loss of the bacterial MC during antibiotics treatment.

FEMS7-2560
Pathogenicity

UNCOMMON VIRULENCE POTENTIAL IN ST-14 KPC-3 KLEBSIELLA PNEUMONIAE: A HIGH RISK CLONE IN PORTUGAL

C. Caneiras[1], L. Lito[2], J. Melo-Cristino[2,3], A. Duarte[1]
[1]Research Institute for Medicines iMed.ULisboa - Faculty of Pharmacy - University of Lisbon, Department of Microbiology and Immunology, Lisboa, Portugal
[2]Centro Hospitalar Lisboa Norte, Laboratory of Microbiology, Lisboa, Portugal
[3]Institute of Molecular Medicine - Faculty of Medicine - University of Lisbon, Institute of Microbiology, Lisboa, Portugal

Backgrounds

The emergence of KPC-type carbapenemases in *Klebsiella pneumoniae* poses a serious threat to public health worldwide and have recently undergone a relevant increasing prevalence in Portugal. Studies regarding the *K. pneumoniae* identification of virulence markers and high-risk clones are critical for the development of novel diagnostic methods and new therapeutic strategies.

Objectives

The aim of this study was the identification of virulence markers and characterization of the clonal relationship among multidrug resistant *Klebsiella pneumoniae* isolates producing KPC-3 carbapenemases since 2009, in Portugal.

Methods

This study included 27 representative clinical isolates of *K.pneumoniae* KPC-3 producers collected in a tertiary hospital centre in Lisboa, Portugal, between 2009 and 2013. Antimicrobial susceptibility testing was performed by disk diffusion and the results were interpreted according to CLSI and EUCAST guidelines. Genes encoding other β-lactamases including OXA, NDM, CTX-M, TEM, SHV, DHA, FOX, and CMY were screened by PCR and confirmed by sequencing. The isolates were also screened for gene markers of virulence factors: *K2A, fimH, mrkD$_{V1}$, mrkD$_{V2-4}$, khe, rmpA, magA,* and *iucC* by PCR amplification.The clonal relationship was evaluated by M13 fingerprinting and multilocus sequence typing (MLST).

Conclusions

We firstly report an uncommon and concerning overlapping of multidrug-resistance and accumulation of virulence genes in the prevalent (>80%) ST-14 clone identified. The combination of the KPC-3 gene with virulence genes as K2 capsular serotype, fimbrial adhesins, haemolysin, and aerobactin - a bacterial iron chelating agent, can constitute a serious threat, especially for vulnerable populations and may further exacerbate infections caused by this pathogen.

FEMS7-0336
Pathogenicity

RIBOSWITCH REGULATION OF METHIONINE METABOLISM AND VITAMIN B12 UPTAKE IN MYCOBACTERIA – A ROLE IN PATHOGENESIS?

T. Kipkorir[1], G. Mashabela[1], V. Mizrahi[1], D.F. Warner[1]
[1]University of Cape Town, Pathology and Institute of Infectious Disease and Molecular Medicine, Cape Town, South Africa

Backgrounds

Mycobacterium tuberculosis (MTB) is thought to recognize fluctuations in specific environmental stimuli within the host, and to respond with corresponding alterations to gene expression and metabolism. Accordingly, the MTB genome contains a large number of regulatory elements which could contribute to this capacity. These include two vitamin B_{12} (B_{12}) riboswitches that have been identified upstream of *metE* – encoding a B_{12}-independent methionine synthase, and *ppe2* – the first gene in a putative operon with the B_{12} biosynthetic genes, *cobQ1* and *cobU*. We showed previously that disruption of the alternative, B_{12}-dependent methionine synthase, MetH, rendered MTB sensitive to B_{12}, presumably via methionine auxotrophy owing to B_{12} riboswitch-dependent suppression of MetE levels.

Objectives

Unlike MTB, MSM is a constitutive B_{12} producer under standard conditions *in vitro*. Therefore, we hypothesized that MetH would be essential in MSM, and that deletion of *metH* would be possible only by *metE* riboswitch inactivation or in a B_{12} deficient background.

Methods

Targeted knock-out of *metH* proved facile in a MSM Δ*cobK* mutant in which *de novo* B_{12} biosynthesis is disrupted. Curiously, however, the double Δ*metH* Δ*cobK* deletion mutant was not sensitive to exogenous B_{12}. Comparative genomic analyses identified additional B_{12} riboswitches controlling two separate BtuFCD-type B_{12} transporters that are present in MSM but not MTB.

Conclusions

These findings suggest that B_{12}-dependent metabolism in MSM is regulated both at the level of cofactor transport and enzymatic function, thus identifying B_{12} uptake as one potential point of departure in the evolution of the pathogenic MTB from the most recent common mycobacterial ancestor.

FEMS7-2805
Pathogenicity

CANDIDA ALBICANS IS ABLE TO ALTER ENTEROCYTE TIGHT JUNCTIONS THAT ENHANCES ITS TRANSLOCATION THROUGH THE GUT BARRIER

A. Loiselet[1,2], F. Bon[1], A. Ducreux[1], C. d'Enfert[3], B. Hube[4,5], F. Dalle[1,2]
[1]UMR A PAM Univ Bourgogne-Franche-Comte, UFR Sciences de Santé- Bât B3, Dijon, France
[2]Centre Hospitalier Universitaire, Service de Parasitologie Mycologie, Dijon, France
[3]Institut Pasteur- INRA, Unité Biologie et Pathogénicité Fongiques, Paris, France
[4]Leibniz Institute for Natural Product Research and Infection Biology—Hans Knoell Institute, Department of Microbial Pathogenicity Mechanisms, Jena, Germany
[5]University Hospital Jena, Center for Sepsis Control and Care, Jena, Germany

Backgrounds

C.albicans is commensal yeast of the mucosa in healthy humans that can be responsible for disseminated candidiasis, mainly originating from the gut. Deciphering the cellular and molecular mechanisms of the interaction of *C.albicans* with enterocytes is necessary to better understand the basis of commensalism and pathogenicity of the yeast and to improve the management of disseminated candidiasis. Different routes of tissue invasion by *C.albicans* are reported, partly due to differences in the structure of the epithelial layer. The intestinal epithelium consists in a monolayer of enterocytes where adjacent cells are jointed each other through cell junctions. Among those, tight junctions (TJ) ensure integrity and impermeability of the intestinal barrier, limiting invasion of the gut by *C.albicans.*

Objectives

The aim of our work is to investigate the impact of *C. albicans* interaction upon the integrity of the intestinal barrier.

Methods

Permeability of the intestinal barrier is investigated measuring the transepithelial electrical resistance of Caco-2 cell monolayers interacting with *C. albicans*. The organization of the TJ protein complex is studied by (i) monitoring their cellular localization using microscopy and (ii) their cellular distribution by using western blotting and Nano-HPLC. Molecules of the fungus responsible for these cellular alterations are currently under investigation.

Conclusions

Candida albicans is able to disorganize the TJ protein complex, altering the integrity of the intestinal barrier. As a consequence, *C. albicans* enhances its translocation through the gut barrier. Better understanding the first steps of *Candida* invasion will help to find target molecules to prevent its dissemination during severe candidiasis.

FEMS7-1514
Pathogenicity

SCREENING DISRUPTORS OF THE TOXIN–ANTITOXIN COMPLEX IN STAPHYLOCOCCUS AUREUS

Y. Ota[1], T. Miyamoto[1], A. Yokota[2], S. Tsuneda[1], N. Noda[2]
[1]Waseda University, Department of life science and medical bioscience, Tokyo, Japan
[2]National Institute of Advanced Industrial Science and Technology, Biomedical research institute, Tsukuba, Japan

Backgrounds

Toxin–antitoxin (TA) modules, which are implicated in microbial cell growth and survival, are widespread in prokaryotic cells. These modules comprise toxins, which are harmful to the host cell and antitoxins, which commonly neutralize the cognate toxins by forming these TA complexes. Therefore, TA complexes are considered promising targets for the development of novel antimicrobial agents to combat the rapid spread of antibiotic resistance in major human pathogens such as *Staphylococcus aureus*.

Objectives

Herein, we aimed to identify new antimicrobial peptides directly targeting the MazEF module, a typical TA module, in *S. aureus* by using a high-throughput screening method combining the phage display technique, massively parallel sequencing, and a continuous fluorometric assay.

Methods

We enriched candidate peptides with a high affinity for the MazF toxin by using phage display technology involving repetitive biopanning. The peptide amino acid sequences were comprehensively determined using massively parallel sequencing. Thereafter, we utilized a continuous fluorometric assay to assess the ability of the peptides to liberate the MazF toxin in the presence of the MazE antitoxin.

Conclusions

Using biopanning followed by massively parallel sequencing, we identified several peptides that specifically bind to the MazF toxin, and subsequently, using a continuous fluorometric assay, we revealed that some peptides overcome the inhibitory effect of the MazE antitoxin on the MazF toxin *in vitro*. Our method can be readily applied in the discovery of disruptors of other TA systems using direct binding mechanisms.

FEMS7-0417
Pathogenicity

SALMONELLA TYPHIMURIUM REQUIRES SOPB AND SIFA TO SURVIVE INTRACELLULARLY IN A SCV-LIKE COMPARTMENT IN DICTYOSTELIUM DISCOIDEUM

C. Valenzuela[1], *A. Sabag*[1], *Í.M. Urrutia*[1], *M. Varas*[2], *C.A. Santiviago*[1]
[1]*Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Santiago, Chile*
[2]*Universidad de Chile, Facultad de Ciencias, Santiago, Chile*

Backgrounds

Salmonella survival within eukaryotic cells is explained, in part, by its ability to establish an intracellular compartment known as the *Salmonella* containing vacuole (SCV). To this end, *Salmonella* exploits several effector proteins secreted by type-three secretion systems $T3SS_{SPI-1}$ and $T3SS_{SPI-2}$. We recently reported a role for effector proteins SopB and SifA in the survival of *Salmonella* Typhimurium in *Dictyostelium discoideum*, and the presence of wild-type bacteria in a vacuolar compartment within this amoeba.

Objectives

We evaluated whether the defect in intracellular survival of Δ*sopB* and Δ*sifA* mutants is explained by their inability to survive within SCV-like vacuoles.

Methods

We performed infections of *D. discoideum* expressing a VatM-GFP fusion using bacterial strains expressing the red fluorescent protein mCherry, and analyzed infected cells by confocal laser microscopy. VatM is a vacuolar ATPase used as marker of SCV membranes in other host cells.

Conclusions

According to our results, viable wild-type bacteria were detected laying within VatM-GFP positive vacuoles up to 6 h of infection. In the case of Δ*sopB* and Δ*sifA* mutants, only a few bacteria were detected within VatM-GFP positive vacuoles at 3 h of infection, and most *D. discoideum* cells contained remnants of killed bacteria. Altogether, our results suggest that *S.* Typhimurium resides in a SCV-like compartment in *D. discoideum* and requires effectors SopB and SifA to avoid degradation by this amoeba. We are currently characterizing the composition of the SCV-like compartment in *D. discoideum*.

Supported by FONDECYT grants 1140754 and 1171814, and CONICYT fellowships 21140615, 22140758 and 21150005.

FEMS7-3014
Pathogenicity

PSEUDOMONAS PROTEGENS AND PSEUDOMONAS CHLORORAPHIS: SWITCH BETWEEN ROOT- AND INSECT-ASSOCIATED LIFESTYLES

P. Vesga[1], P. Flury[1], M. Péchy-Tarr[2], C. Keel[2], M. Maurhofer[1]
[1]Institute of Integrative Biology- Federal Institute of Technology Zurich ETHZ, Plant Pathology, Zurich, Switzerland
[2]University of Lausanne, Fundamental Microbiology, Lausanne, Switzerland

Backgrounds

Insect pests are a major agricultural problem and very difficult to control. Root-colonizing fluorescent pseudomonads are known for their ability to promote plant growth and to protect plants against soilborne fungal pathogens, but some of them belonging to the species *Pseudomonas protegens* and *P. chlororaphis* additionally have insecticidal activity. They can colonize the gut of insect larvae and invade the hemocoel causing systemic infection and death. These bacteria produce an insect toxin termed Fit, which contributes to insecticidal activity.

Objectives

To answer the question whether *P. protegens* and *P. chlororaphis*, which were so far thought to be mostly associated with plants and soil, are naturally associated with insects.

Methods

In order to answer this question, bacteria were isolated from insects collected from the field. Isolates were assessed for presence of the Fit insect toxin gene and insecticidal activity and were phylogenetically characterized. So far, we found that Fit-harbouring pseudomonads are common in different insect species, which indicates that insects are an additional ecological niche for *P. protegens* and *P. chlororaphis*. Currently, we are performing a RNA-sequencing analysis with *P. protegens* CHA0 to determine the genes it needs to switch between a root- and an insect-associated lifestyle. In addition, we have demonstrated, in insect backgrounds, the expression of known insecticidal factors as well as factors involved in suppression of plant pathogens and competition.

Conclusions

Altogether, this knowledge will contribute to a better understanding of *Pseudomonas*-insect interactions and to develop future biocontrol strategies for insects on top of diseases.

FEMS7-2799
White Biotechnology

EVOLUTIONARY HISTORY OF FUNGAL VERSATILE-LIPASES FROM THE ORDER AGARICALES

J. Barriuso[1], *M.J. Martínez*[1]
[1]*Centro de Investigaciones Biológicas CIB-CSIC, Environmental Biology, Madrid, Spain*

Backgrounds

Lipases (EC 3.1.1.3) and sterol esterases (EC 3.1.1.13) are thoroughly used in industrial applications. Among fungi some extracellular proteins combine properties of both enzymes, and there are known as “Versatile carboxylic ester hydrolases” (abH03.01). Agaricales is the largest clade of mushroom-forming fungi and one of the most ancient orders, comprising wood saprophytes with biotechnological interest. On the other hand, the *in silico* mining of fungal genomes and metagenomes has been shown as an alternative for searching novel lipases, while exploring the evolutionary history and resurrecting intermediate ancestral forms of enzymes can help to explain the mechanistic basis of enzymes function and disclose new functionalities.

Objectives

In this work we explored the presence of genes encoding putative “Versatile lipases” from the order Agaricales. We reconstructed the molecular evolution of these enzymes and inferred the sequence of their ancestral intermediate forms. The potential properties of the candidates are discussed on the basis of their three-dimensional (3D) model structure, the presence and hydrophobicity of the lid, and the substrate binding tunnel.

Methods

We used conserved motifs, sequence and phylogenetic analyses, and three-dimensional modeling to look for candidate genes in public fungal genomes. Moreover, we reconstructed the molecular evolution of these enzymes using novel phylogenetic approaches (PAML4.8).

Conclusions

The evolutionary history of the putative lipases revealed an increase on the length and hydrophobicity of the lid region, as well as in the size of the substrate binding pocket, during evolution time. These facts suggest the enzymes’ specialization towards certain substrates and their subsequent loss of promiscuity.

FEMS7-0277
White Biotechnology

LESS IS MORE: HYDROLYSIS OF POLYESTERS IS ENHANCED BY A TRUNCATION OF AN ESTERASE

A. Biundo[1], R. Doris[2,3], G. Steinkellner[4], K. Gruber[5]
[1]Polymer and Environmental Biotechnology, Austrian Centre of Industrial Biotechnology, Tulln, Austria
[2]Institute for Environmental Biotechnology, Department of Agrobiotechnology- IFA-Tulln, Tulln, Austria
[3]Polymers and Environmental Biotechnology, Austrian Centre for Industrial Biotechnology, Tulln, Austria
[4]Austrian Centre of Industrial Biotechnology, Austrian Centre of Industrial Biotechnology, Graz, Austria
[5]Institute of Molecular Bioscience, University of Graz, Graz, Austria

Backgrounds

Polymers frequently used for one-way applications like packaging are preferably biodegradable, albeit non-biodegradable polyesters are mostly used. Biodegradability of the aliphatic/aromatic copolyester poly(butylene adipate-*co*-terephthalate) (PBAT) has been investigated, showing biological decomposability under composting conditions. However, little is known about its anaerobic hydrolysis while large amount of food packaging ends up in biogas plants. The enzyme EstA from *Clostridium botulinum* (Cbotu_EstA) actively hydrolyzed PBAT while it failed to act on polyethylene terephthalate (PET). Yet, enzymes would allow mild decomposition of widely used PET enabling recycling of the monomeric building blocks.

Objectives

The enhancement of the hydrolase activity with regard to polyester hydrolysis was carried out by fusion of hydrophobic domains, improving the biocatalyst adsorption on the hydrophobic polymer surface, or by substitution of specific residues, enlarging the active site of the enzyme. The deletion of the Cbotu_EstA N-terminal domain can satisfactory combine both approaches.

Methods

The 3D structure analysis of Cbotu_EstA revealed the presence of an N-terminal domain covering the lid structure and a hydrophobic patch. Chemoluminescence and HPLC analysis determined the adsorption onto hydrophobic surfaces and the hydrolyzing activity of the enzyme, respectively. Furthermore, analysis of the kinetic constants made the catalytic activity investigation of the two forms of the enzyme possible.

Conclusions

Surface engineering successfully produced a highly active Cbotu_EstA variant which was able to hydrolyze PET. Truncation of the N-terminal domain of Cbotu_EstA improved the adsorption of the enzyme on hydrophobic polyester surfaces and enhanced their hydrolysis eight times more compared to the wild-type enzyme, based on released monomers quantification.

FEMS7-3278
White Biotechnology

CARBOXYLATE PLATFORM CHEMICALS AND BIOFUELS FROM BIOWASTE

E. Hakalehto[1,2], S. Schwede[3], R. Laatikainen[4], E. den Boer[5], L. Piiroinen[1]
[1]Finnoflag Oy, P.O. Box 262, 70101 Kuopio, Finland
[2]Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland
[3]School of Business, Society and Engineering, Mälardalen University, Västerås, Sweden
[4]School of Pharmacy, University of Eastern Finland, Kuopio, Finland
[5]Wrocław University of Science and Technology,
Department of Environmental Protection Engineering, Wroclaw, Poland

Backgrounds

A significant share of the fuels and chemicals used in the economy could be replaced by substances produced from biomass sources. Recent international and European development strategies attribute high priority to the growth of bioeconomy sector. A radical change of production, consumption, processing, storage, recycling and disposal of biological resources is needed, in order to cope with an increasing global population, rapid depletion of many resources, increasing environmental pressures and climate change.

Objectives

Micro-organisms provide tools for circulating the substances in the industrial ecosystems. This paper focuses on the production of carboxylic platform chemicals from industrial and municipal kitchen biowaste using non-aceptic conditions. Verification of this technology has been done based on pilot tests in 3 regions – in Finland, Poland and Sweden.

Methods

The biorefinery technology was implemented in a pilot scale with effective reactor volumes of 200-300 L under anaerobic and microaerobic conditions. The production organisms belonged to the genera *Clostridium* and *Klebsiella*. Selected biowaste streams were subjected to enzymatic pretreatment prior to the inoculation.

Conclusions

The results showed high conversion rates achieved from mixed substrates. The dominating end products were volatile fatty acids (VFAs), ethanol and CO_2. Lower levels of other gases, such as H_2, CH_4 and H_2S, were formed. The conversion rates of glucose to the main fermentation products reached up to 0.81 mol/mol. Valeric acid was one of the main VFA products from the potato waste carbohydrates, whereas in trials on chicken litter, the valerate derived mostly from the amino acids of the biowaste proteins.

FEMS7-1219
White Biotechnology

OLEOGELS ELABORATION USING LIGNIN SOLUBILIZED BY STREPTOMYCES FROM AGRICULTURAL RESIDUES

A. Blánquez[1], J. Rodríguez[2], F. Guillén[2], S. Morales[2], C. Valencia[3], J.M. Franco[4], M.E. Arias[2], M. Hernández[2]
[1]University of Acalá, Dpto. Biomedicine and Biotechnology, Alcalá de Henares, Spain
[2]University of Acalá, Department of Biomedicine and Biotechnology, Alcalá de Henares, Spain
[3]University of Huelva, Department of Chemical engineering-
Physical Chemistry and Materials Science, Huelva, Spain
[4]University of Huelva,
Department of Chemical engineering- Physical Chemistry and Materials Science, Huelva, Spain

Backgrounds

Lignin is an aromatic biopolymer traditionally considered a waste product in industrial processes and agricultural practices causing serious pollution problems. At present, lignin is considered a renewable biopolymer for biotechnological applications. The suitability of *Streptomyces* to solubilize lignin from grasses residues, instead of to mineralize this polymer, could be a good strategy for biotechnological purposes.

Objectives

The aim of this work is to set up the optimal conditions to solubilize lignin from agricultural residues using selected *Streptomyces* strains to be applied for oleogels elaboration.

Methods

Different *Streptomyces* strains were cultured for 7 days under Solid-State Fermentation conditions (SSF) on agricultural residues. Transformed substrates were extracted with water or 0.1 M NaOH and extracts were acidified with HCl. Finally, water or alkali-extracted lignin were gravimetrically estimated. Moreover, xylanase, mannanase and laccase activities were determined along the time course of growth. Selected strains were also cultured under SSF conditions supplemented with radicals promoting agents such as quinones and Fe^{3+}-EDTA. Prior to this, the Fe^{3+} content of substrates (60 ± 3 µg g^{-1}) were determined by Atomic Absorption Spectrophotometry. For oleogels preparation alkali-lignin was modified with 1,6-hexamethylene diisocyanate.

Conclusions

An increase in 10-20% of alkali-lignin yield was achieved with selected strains compared to that obtained from uninoculated substrates.

Under radical producing conditions, the colonization ability of strains was not affected being the yield of water-extracted lignin 20 % higher than that obtained in non-supplemented cultures with radical production promoters.

Preliminary assays carried out with oleogels elaborated with alkali-lignin showed suitable thermal resistance and rheological characteristics.

FEMS7-3175
White Biotechnology

CONSTRUCTION AND CHARACTERIZATION OF MICROBIAL CONSORTIA FOR KERATIN DEGRADATION

D. Kang[1], S. Jacquiod[2], J. Herschend[1], S.J. Sørensen[1]
[1]University of Copenhagen, Department of Biology, Copenhagen, Denmark
[2]INRA, UMR 1347 Agroécologie, Dijon, France

Backgrounds

Europe, especially Denmark, heavily relies on import of soy protein for feed in its animal production. Keratins refer to a group of insoluble, tough and recalcitrant protein material generated in large quantities as a waste-product in commercial slaughterhouses, originating from bristles and hooves. Utilization of microbial consortia can play a crucial role as natural biodegradation process for keratin. It can be transformed into more valuable feedstock through the release of small peptides and amino acids by microbial degradation.

Objectives

Identify microbial communities for keratin degradation and construction of stable microbial consortia for industrial applications.

Methods

A soil-born microbial consortium was enriched on keratin as the sole carbon source in sequential batch cultivations at room temperature and characterized by application of next generation sequencing and specific keratin degradation assays.

Conclusions

During six enrichment cycles, the procedure selected for stable and efficient keratin degrading microbial consortia, mainly constituted by members of bacteroidetes and proteobacteria phyla. For the sake of industrial applications, we used a dilution-based method to reduce the diversity and isolate key component strains involved in efficient keratin degradation, while excluding potential cheaters and ease of controllable outputs. The consortia were structurally stable with the co-existence of four major microbes, comprising aerobic bacterial genera *Chryseobacterium*, *Stenotrophomonas*, *Pseudochrobactrum, Acinetobacter*. Residual substrates proportion is similar when comparing with using the microbial consortium without dilution and approximate 20% after five days. This work has potential applications for a vast range of areas including food and feed, fisheries, biotechnologies and agriculture.

FEMS7-0357
White Biotechnology

OPTIMIZATION OF A FAST AFFINITY ADSORPTION PURIFICATION METHOD OF FUNCTIONAL LPMOS

S. Valenzuela[1], *G. Ferreres*[1], *G. Margalef*[1], *M. Rodríguez-Astigarraga*[1], *F.I.J. Pastor*[1]
[1]*University of Barcelona, Department of Genetics- Microbiology and Statistics, Barcelona, Spain*

Backgrounds

The recently discovered LPMOs are crucial enzymes for the deconstruction of recalcitrant macromolecules, including lignocellulosic and chitin polymers, thus represent a promising component of industrial cocktail enzymes for degrading biomass. The LPMOs are metalloenzymes that oxidize glycosidic bonds and the catalysis requires the binding of an active oxygen molecule to the copper atom of the enzyme for the oxidative depolymerization of the substrate. Depending on the type of LPMO, the products released are gluco-oligosaccharides that are oxidized either at the reducing (C1) or in both, the reducing (C1) and the non-reducing (C4) end (3).

Objectives

To develop a simple and low-cost method for recombinant LPMOs purification.

Methods

We have cloned two orfs encoding for putatives LPMOs from *Streptomyces ambofaciens*, which is a soil bacterium industrially exploited for the production of antibacterial chemicals. We established a purification method for these enzymes based on the ability of the recombinant LPMOs to bind polysaccharides. In addition, one of the produced LPMOs, named SamAA10C was tested against a variety of cellulosic substrates, including for the first time for an LPMO, bleached pulps used in the paper industry.

Conclusions

We developed a simple and fast method to purify LPMOs in a single step without using any chromatographic column or periplasmic preparation, considerably reducing time used in others studies for the purification step. The purification method introduced for the LPMOs could be a cheap solution, to use these enzymes in industrial applications, and the activity of SamAA10C show the potential of LPMOs in biomass upgrading.

FEMS7-1225
Biotechnology / Synthetic Biology / Systems Biology

FROM GRASS TO GAS: MICROBIOME DYNAMICS OF GRASS BIOMASS ACIDIFICATION UNDER MESOPHILIC AND THERMOPHILIC TEMPERATURES

C. Abendroth[1], C. Simeonov[2], J. Peretó[1], O. Antúnez[3], R. Gavidia[3], O. Luschnig[4], M. Porcar[1]
[1]Institute for Integrative Systems Biology I2SysBio- University of Valencia-CSIC, Biotechnology, València, Spain
[2]Robert Boyle Institut e.V., Anaerobic Digestion, Jena, Germany
[3]Universitat de València, Servicio Central de Soporte a la Investigación Experimental, València, Spain
[4]Bio H2 Umwelt GmbH, Anaerobic Digestion, Jena, Germany

Backgrounds

Separating acidification and methanogenic steps in anaerobic digestion processes can help to optimize the process and contribute to producing valuable sub-products such as methane, hydrogen and organic acids. However, the full potential of this technology has not been fully explored yet.

Objectives

The aim of this work was to finely characterize the acidification as one of the most important phases of the biogas production process.

Methods

To assess the underlying fermentation process in more detail, we applied for the first time a combination of high throughput sequencing and proteomics on the acidification step of plant material (grass) at both mesophilic and thermophilic temperatures (37 ºC and 55 ºC, respectively).

Conclusions

High strength liquor from acidificated grass biomass exhibited a low biodiversity and was proved very different depending on the temperature. It was dominated by Bacteroidetes and Firmicutes at 37 ºC, and by Firmicutes and Proteobacteria at 55 ºC.

In the methane stage, *Methanosaeta*, *Methanomicrobium* and *Methanosarcina* proved very sensitive to environmental changes as their abundance in the seed sludges dropped drastically after transferring the seed sludges from the respective reactors into the experimental set up. Further, an increase in Actinobacteria coincided with reduced biogas production in the end of the experiment.

Temperature proved a key parameter affecting both taxonomic and proteomic profiles, which were strikingly diverse at 37 ºC and at 55 ºC. Our results suggest that screening approaches targeting *Methanosaeta, Methanomicrobium* and *Methanosarcina* could be useful diagnosis tools as indicators of environmental changes as for example temperature or oxidative stress.

FEMS7-1433
Biotechnology / Synthetic Biology / Systems Biology

GENOME-SCALE METABOLIC RECONSTRUCTION OF ARTHROSPIRA PLATENSIS PCC9108 AS A TOOL TO IMPROVE ITS TECHNOLOGICAL AND NUTRITIONAL PROPERTIES

L. Agudo[1], *J.L. García*[1], *J. Nogales*[1]
[1]*Centro de Investigaciones Biológicas CIB-CSIC, Environmental Microbiology, Madrid, Spain*

Backgrounds

Arthrospira (Spirulina) *platensis* is one of the most valuable cyanobacteria and widely used as source of added-value compounds, and as feeding due its nutraceutical properties. Elusive so far to genetic manipulation, the improvement of its natural properties by metabolic engineering approaches is challenging. Nonetheless, the increasing development of the metabolic reconstruction field allows the systematically analysing of bacterial metabolic capabilities, at genome-scale. Hence, system biology will becomes a powerful tool to identify new rational ways of improving the biotechnological properties of Spirulina.

Objectives

Analyzing the metabolic properties of *Arthrospira platensis* PCC9108 through Systems Biology approaches.

Methods

We sequenced and annotated the genome of *Arthrospira platensis* PCC9108 using PacBio and RAST server, respectively. Furthermore, we reconstructed a manually curated, full compartmentalized, and mass and charge balanced genome-scale metabolic reconstruction, based on well-known procedures (Nogales, 2014). Finally, we analyze the metabolic features of Spirulina, emphasizing on light's metabolism by means of Constraint-Based Reconstruction and Analysis (COBRA) approach (Lewis et al., 2012).

Conclusions

Based on the genome annotation and metabolic knowledge available, we have constructed one of the most complete cyanobacterial genome-scale metabolic models (*i*LA649). *i*LA649 includes 649 genes, 788 reactions and 749 metabolites. In addition *i*LA649 accounts for a detailed modelling of the light-harvesting as well as a large array of condition-specific biomass objective functions. The potential of *i*LA649 as computational tool for Spirulina based photoenginiering endeavours will be discuss.

Lewis NE et al. (2012). *Nat Rev Microbiol* 10(4): 291–305

Nogales J (2014). Humana Press, New York, pp 1–25

FEMS7-1545
Biotechnology / Synthetic Biology / Systems Biology

NEW GLYCOSIDES FROM A STREPTOMYCES SP. DISCOVERED BY A COMBINATION OF BIOASSAY AND CHEMICAL SCREENING

J.S. Ahn[1], S. Son[1], J. Mina[1], S.K. Ko[1], J.H. Jang[1]
[1]Korea Research Institute of Bioscience and Biotechnology, Anticancer Agent Reseach Center, Cheongju, Republic of Korea

Backgrounds

A number of bacterial metabolites are currently used as pharmaceutically important compounds, and they continue to capture a significant part of new molecular entities of FDAs-approved drugs, demonstrating a continuous search for novel bacterial metabolites is still valuable.

Objectives

In that context, we have screened new bioactive small molecules produced by actinomycetes strains isolated from the soil samples collected from Ulleung Island, a small volcanic island in the Korean East Sea, contains richly diverse microorganisms.

Methods

The cultured extracts of isolated actinomycetes were screened through dereplication strategy based on the results from *in vitro* indoleamine 2,3-dioxygenase (IDO) inhibition assay and HPLC-PDA-MS analysis.The chemical structures were elucidated by detailed NMR and MS spectroscopic analyses. And absolute configurations of the sugar units were determined by the magnitudes of the coupling constants, NOESY correlations, chemical derivatization, and optical rotation measurements.

Conclusions

In the course of screening, we could isolate six secondary metabolites including three new rare glycosides inhibiting IDO enzyme activity from *Streptomyces* sp. KCB13F030. The isolated compounds showed anti-proliferative activities against cancer cell lines and effects on autophagy by observing GFP-LC3 puncta formation in GFP-RFP-LC3 stably expressing HeLa cells. Feeding experiments using resveratrol as a substrate afforded two new mono-glycosylated resveratrols, suggesting the talosylation potential of the strain.

FEMS7-2944
Biotechnology / Synthetic Biology / Systems Biology

FERMANTATION OF WHEY POWDER TO BIOETHANOL BY VITREOSCILLA HEMOGLOBIN EXPRESSING E. COLI

M.Y. Akbas[1], *T. Sar*[1], *G. Seker*[1], *A.G. Erman*[1]
[1]*Gebze Technical University, Molecular Biology and Genetics, Gebze-Kocaeli, Turkey*

Backgrounds

Bioethanol is the most widely used biofuel for transportation because it is nontoxic and renewable. Cheese whey an inexpensive and abudant food processing waste, could be a promising feedstock for bioethanol production. *Vitreoscilla* Hemoglobin (VHb), the best characterized prokaryotic hemoglobin, enhances respiration under microaerobic conditions. Engineering of bacteria to express VHb has resulted in increased production of many industrially important products.

Objectives

The possible enhancement of ethanol production from whey powder containing media by using *Vitreoscilla* hemoglobin expressing E.coli strains was investigated.

Methods

Ethanol production from whey powder was investigated by using free and immobilized ethanologenic *E. coli* strain FBR5 and bacterial hemoglobin (*Vitreoscilla* hemoglobin, VHb) expressing strain TS3 in different concentrations of whey powder containing fermentation media.

Conclusions

The highest ethanol production was determined by combination of VHB with immobilization by using a medium with an intermediate concentration of lactose. Immobilization of VHb-expressing ethanologenic *E. coli* was found to be a useful approach for ethanol production from whey powder.

FEMS7-2945
Biotechnology / Synthetic Biology / Systems Biology

PYROLYZED BACTERIAL CELLULOSE AS AN ANODE MATERIAL IN LITHIUM-ION BATTERIES

M.Y. Akbas[1], T. Sar[1], B. Dursun[2], G. Seker[1], A.G. Erman[1], R. Demir-Cakan[3]
[1]Gebze Technical University, Molecular Biology and Genetics, Gebze-Kocaeli, Turkey
[2]Gebze Technical University, Material Science and Engineering, Gebze-Kocaeli, Turkey
[3]Gebze Technical University, Chemical Engineering, Gebze-Kocaeli, Turkey

Backgrounds

There is an increasing demand for a flexible, non-toxic and facile production method for rechargeable batteries used in portable electronic devices. Lithium-ion batteries are one of the appropriate rechargeable batteries due to their higher energy density.

Graphite is widely used as an anode material in lithium-ion batteries. However, graphite anodes have low charge / discharge current density and relatively low capacity. Thus, to find alternative high capacity anode materials at low cost have attracted great attention. Biological materials can be served as attractive carbon materials for energy storage. Among the biological materials, bacterial cellulose (BC) is produced by a bacterium *G.xylinus*. BC which is porous, economic and easy to produce material in large amounts.

Objectives

The potential use of pyrolyzed bacterial cellulose was investigated as an anode material in lithium-ion batteries.

Methods

BC samples were produced and pyrolyzed to yield carbonaceous material under Ar atmosphere at different temperatures. The electrochemical performances of pyrolyzed BCs were evaluated over the 300 cycles.

Conclusions

Pyrolyzed BCs samples showed stable discharge capacities. BC could be used as a potential anode material in Lithium-ion batteries.
This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK, 115M390).

FEMS7-0531
Biotechnology / Synthetic Biology / Systems Biology

EFFECT OF CONTACT-DEPENDENT GROWTH INHIBITION (CDI) ON BIOFILM FORMATION OF E.COLI 25922

M.H. Akyıl[1], N. Cihangir[1], S. Genç[1], M. Sayın[1]
[1]Hacettepe University, Biology, Ankara, Turkey

Backgrounds

Contact-dependent growth inhibition CDI is a mechanism of inter-bacterial competition, it has been firstly observed in *E.coli* isolate EC93. CDI is mediated by the CdiB/CdiA two partner secretion proteins. The CdiA effector extends from the surface of CDI(+) inhibitor cells, binds to receptors on neighbouring bacteria and delivers a toxin domain derived from its C-terminal region (CdiA-CT). The C-terminal domain typically possesses toxic nuclease activity, whereas the N-terminal domain appears to control toxin transport into target bacteria. Biofilm is an assemblage of microbial cells that is irreversibly associated with a surface and enclosed in a matrix of primarily polysaccharide material. In previus studies it was shown that disruption of the cdi locus abrogates biofilm formation.

Objectives

We aimed that compare biofilm formation *E.coli* 25922 (CDI^+)with *E.coli* 25922 ΔcdiA (CDI^-).

Methods

In silico analysis of *E.coli* 25922 genome revealed that the WP_038429283 loci contain characteristic features of a CDI locus. Datsenko-Wanner's "One step inactivation" protocol were used to obtain *E.coli* 25922 ΔcdiA. Biofilm formation assays were performed following a previously described method (Danese et al. 2000) with few modifications.

Conclusions

We determined that there are positive correlation between CDI gene and biofilm formation. It is estimated that on adhesion step, CDI has an effective rol. Consequently CDI gene can be considered as a control mechanism that induces biofilm.

FEMS7-3058
Biotechnology / Synthetic Biology / Systems Biology

ANTI-BIOFILM ACTIVITY OF THE METABOLITES OF STREPTOMYCES ISOLATES AGAINST ENTEROBACTERIACEAE

M.H. Akyıl[1], *N. Cihangir*[1]
[1]*Hacettepe University, Biology, Ankara, Turkey*

Backgrounds

More than 70% of naturally occurring antibiotics have been isolated from different genus of Actinomycetes. As with the other Actinobacteria, Streptomycees are Gram-positive, and have genomes with high GC content. Streptomyces produce complex secondary metabolites. These metabolites are very efficient and useful for the treatment of bacterial disease, cancer and biofilm formation. The Enterobacteriaceae are a large family of Gram-negative bacteria that includes many pathogens, *Salmonella, Escherichia coli, Yersinia pestis, Klebsiella*, and *Shigella.*

Objectives

The main purpose of this study is to investigate the antibiofilm effect of metabolites that isolated from various Streptomyces aganist; *E.coli* ATCC® 25922, *Salmonella bongori* ATCC® 43975, *Shigella flexneri* ATCC® 12022, *Klebsiella pneumoniae* ATCC® 700603.

Methods

We produced various metabolites from Streptomyces that we isolated from soil. 20 different Streptomyces was used for this research. Initially we investigated animicrobial effects of these metablites against determined microorganisms. Finally we studied the effects of these metabolites on *E.coli* ATCC® 25922, *Salmonella bongori* ATCC® 43975, *Shigella flexneri* ATCC® 12022, *Klebsiella pneumoniae* ATCC® 700603 biofilm formation. The antibacterial activity of pure isolates was determined by cross-streak method on Mueller Hinton Agar (MHA). Biofilm formation assays were performed following a previously described method (Danese et al. 2000) with few modifications.

Conclusions

We determined that same metabolites has antimicrobial quality and also antibiofilm activity but some of them is ineffective on biofilm formation. We estimated that this result is because of the metabolites mechanism of actions or because of biofilm formation speed.

FEMS7-1286
Biotechnology / Synthetic Biology / Systems Biology

EFFICACY OF BCG PASTEUR AND MTBVAC IN IMMUNOTHERAPY OF BLADDER CANCER: IN VITRO AND IN VIVO MODEL

S. Alvarez-Arguedas[1], *C. Martín*[1], *N. Aguiló*[1]
[1]*Mycobacterial Genetic Group,*
Department of Microbiology- Preventive Medicine and Public Health. Universidad de Zaragoza, Zaragoza, Spain

Backgrounds

In 2012, there were globally reported around 430,000 new cases and 165,000 deaths due to bladder cancer. The most commonly used bladder cancer immunotherapy is the current vaccine against tuberculosis called bacillus Calmette-Guerin (BCG) (Brandau, Biom. & Pharm., 2007). In the development of a new tuberculosis vaccine for replacing BCG (Arbués, Vaccine, 2013), we studied the effect of our candidate vaccine MTBVAC on the treatment of bladder cancer.

Objectives

1) Comparison of *in vitro* interaction of MTBVAC and BCG with human established bladder cancer cell lines. 2) Efficacy of MTBVAC and BCG in a murine model of bladder cancer.

Methods

In vitro cultures of established cell lines T24 and J82 (human origin) at different multiplicities of infection (MOI) with BCG Pasteur-GFP or MTBVAC-GFP (these strains constitutively express the GFP). The percentage of infection, cell death and internalization mechanism were studied by Flow Cytometry and Confocal Microscopy. Additionally, we use a murine model of bladder cancer based in MB49-luc (this strain expresses the luciferase enzyme) to compare the survival differences between the treatment with BCG or MTBVAC.

Conclusions

MTBVAC showed a significantly higher capacity of infection at 4 and 7 days compared to BCG Pasteur. Accordingly, MTBVAC also produced a higher cell death of tumor cells than BCG Pasteur. On the other hand, both vaccine strains enter into the tumor cells by macropinocytosis but the organelles that contain them have different pH values. Finally, the survival rate of the mice treated with MTBVAC are significantly higher than those with BCG.

FEMS7-0194
Biotechnology / Synthetic Biology / Systems Biology

A POLY-EXTREMOPHILIC PEROXIDASE FROM A HALOTOLERANT BACTERIUM, KOCURIA PALUSTRIS STRAIN BW

M.A. Amoozegar[1], *M. Siroosi*[1], *K. Khajeh*[2]
[1]*University of Tehran - Tehran - Iran, Biology, Tehran, Iran*
[2]*Tarbiat Modares University, Biology, Tehran, Iran*

Backgrounds

Dye-decolorizing peroxidase is a new superfamily of haem-peroxidases isolated from fungi and bacteria. Dye-decolorizing peroxidases, as a new group of peroxidases, are potent biocatalysts to decolorize various dyes which makes them appropriate options for bioremediation of wastewaters contaminated with dyes

Objectives

In the present work, the peroxidase from a halotolerant bacterium, *Kocuria palustris* strain BW isolated from Badab Sourt spring in northern Iran was partially purified and then, characterized. Also, decolorization activity of the peroxidase was tested on two different dyes, aniline blue and toluidine blue.

Methods

Ammonium sulfate precipitation and thermal treatment methods were used to partial purification of the enzyme. In order to assay peroxidase activity spectrophotometrically, H2O2 was added to the enzymatic reaction mixture contained 50 mM citrate-phosphate buffer (pH 3), 4.5 M NaCl, appropriate amount of enzyme, and 0.5 mM ABTS at 90 °C and the increase in the absorbance at wavelength of 420 nm was followed.

Conclusions

Among 100 different bacterial strains screened for peroxidase activity on a solid culture medium supplemented with guaiacol, one bacterial strain showed reddish-brown color around its colony, the sign of peroxidase activity. According to 16S rRNA gene sequence, the strain showed 100 % similarity to *K. palustris* DSM 11925. The partially purified peroxidase showed poly-extremophilic nature and its optimum activity was at 90 ºC, pH 2.2-3, and 4.5 M NaCl. Decolorization study showed that the peroxidase decolorized both dyes up to 50% at optimum pH of 2.2. The study is the first report on poly-extremophilic peroxidases isolated from a halotolerant bacterium.

FEMS7-2306
Biotechnology / Synthetic Biology / Systems Biology

THE ORPHAN RESPONSE REGULATOR AOR1 FROM STREPTOMYCES COELICOLOR IS A NEW PIECE IN THE PUZZLE OF THE REGULATION NETWORK OF ANTIBIOTIC PRODUCTION

S. Antoraz[1], S. Rico[1], H. Rodriguez[2], M. Díaz[1], R. Santamaría[1]
[1]Salamanca University, Instituto de Biología Funcional y Genómica IBFG, SALAMANCA, Spain
[2]CIC bioGUNE, Molecular recognition and Host-Pathogen interactions, Bizkaia, Spain

Backgrounds

Antibiotic production in *Streptomyces coelicolor* responds to different environmental signals, both physicals (pH, temperature...) and chemicals (nutrients, activators...), through a complex signalling network. Recognisance of these stimuli is performed mainly through Two Component Systems (TCS). Usually, TCSs are composed by a histidine kinase (HK) and a response regulator (RR), forming a single operon in the genome, but also exist HKs and RRs without its partner, the so-called orphans. Presence of orphan genes may serve as a way of increasing the response capacity in the cell, expanding regulatory network's complexity.

Objectives

In this work we try to elucidate the role of one of these orphan regulators, Aor1 (Antibiotic Orphan Regulator), that apparently has great importance in antibiotic production in *S. coelicolor* and differentiation.

Methods

We have obtained the deletion mutant (Δ*SCO2281*) in S. *coelicolor* that allowed us to demonstrate that this orphan RR is involved in the positive regulation of antibiotic production and differentiation. This mutant is drastically affected in endogenous antibiotic biosynthesis of actinorhodin, undecilprodigiosin and calcium dependent antibiotic. We have also evidence that shows a possible relation between this RR and the TCS AbrC. On the other hand, we have demonstrated, by q-RT-PCR, that gene *aor1* is part of a four gene operon (*SCO2279-2282*).

Conclusions

RR Aor1 seems to have an important role in antibiotic production in *S. coelicolor*, and its study adds significant information about this main piece in the puzzle that could serve for unveiling the complex antibiotic regulatory network in *Streptomyces.*

FEMS7-2608
Biotechnology / Synthetic Biology / Systems Biology

ANAEROBACULUM HYDROGENIFORMANS ESTERASE (AHEST): A NEW THERMOSTABLE ESTERASE FOR BIOTECHNOLOGICAL APPLICATIONS

R. Gutierrez[1], J.M. Martins[1], P.S. Kumagai[1], A.M. Castro[2], J.L. Lopes[3], A.P. Araujo[1]
[1]University of São Paulo, Physics Institute of Sao Carlos, Sao Carlos, Brazil
[2]Petrobras, CENPES - Petrobras Research & Development Center, Rio de Janeiro, Brazil
[3]University of São Paulo, Physics Institute, Sao Paulo, Brazil

Backgrounds

Enzymes produced by thermophiles are of interest for biocatalysis because they are stable and active at high temperatures. Additionally, enzymes that are functional in the presence of organic solvents are highly required as biocatalysts, particularly where substrate solubility is limited. Searching for esterases with these attributes, we have identified *in silico* AhEst, a potential esterase from Anaerobaculum hydrogeniformans. The enzyme was produced as a recombinant protein in *E. coli* and used in biochemical and biophysical characterization studies.

Objectives

This study was focused on the evaluation of enzymatic activity and structural stability of the esterase AhEst, in the presence of organic solvents and under increasing temperatures.

Methods

The protein was purified and used for activity assays in the absence or presence of 10% and 20% of ethanol or isopropanol at 25, 45 and 65°C. The activity was measured by the release of *p*-nitrophenol hydrolyzed from the *p*-nitrophenylacetate. Synchroton Radiation Circular Dichroism measurements (SRCD) were used to evaluate the structural stability of AhEst in the same organic solvents described above.

Conclusions

Our results shown that in the presence of ethanol 10% there was no change on enzymatic activity. In isopropanol 10% we observed a significant increase (>20%) in the esterase activity and in 20% of ethanol or isopropanol, AhEst still preserved a high esterase activity (80 to 100%) in all temperatures. SRCD spectra of AhEst showed a high structural and thermal stability in all conditions, agreeing with activity findings. Concluding, AhEst is now available to be used in biocatalytic processes that require extreme conditions.

FEMS7-2150
Biotechnology / Synthetic Biology / Systems Biology

EXPANDING THE GENE REGULATORY NETWORK IN RESPONSE TO OSMOTIC STRESS IN THE HALOPHILE CHROMOHALOBACTER SALEXIGENS

M. Argandoña[1], R. Garcia-Valero[1], A. Tahrioui[1], J.J. Nieto[1], C. Vargas[1]
[1]University of Sevilla, Microbiology and Parasitology, Sevilla, Spain

Backgrounds

Chromohalobacter salexigens is a broad-growing halophilic bacterium considered as a biological model to study osmoadaptation and a cell factory for the production of ectoines, biostabilizing compatible solutes which have many biotechnological applications. To design new metabolic engineering strategies to obtain improved ectoines production strains of *C. salexigens,* a preliminary transcriptional regulatory network (TRN) core was reconstructed by using an integrative approach that combined RNAseq data from wild type strain and different *in silico* analyses. This core network, constituted by 598 genes and 1192 regulatory interactions, was used to analyse molecular regulation events involved in metabolic osmotic stress response and ectoines metabolism

Objectives

To expand the preliminary osmoregulatory network core involving RNA-seq data analysis of two global regulators knockouts: EupR, a response regulator of a two component system that controls both central an ectoines metabolism, and RpoS, a sigma factor involved in central metabolism and osmoadaptation.

Methods

eupR^{-} and *rpoS*$^{-}$ strains were grown at 0.6 and 2.5 M of NaCl (low and high ectoines production conditions respectively). Three replicates of cells were processed for RNA-seq. After analysis of differential gene expression, metabolic and regulatory genes were selected and included in the previous transcriptional regulatory network core.

Conclusions

Subnetwork analysis pointed TFs that were involved in metabolic salinity-dependent response and/or controlled by EupR or RpoS. These results will be used for further development of a hybrid model to connect the metabolic network of *C. salexigens* with its TRN by using a Systems Biology approach

FEMS7-1909
Biotechnology / Synthetic Biology / Systems Biology

VERSATILITY OF THE ALKALINE LACCASE PRODUCED BY STREPTOMYCES IPOMOEAE CECT 3341 FOR BIOTECHNOLOGICAL AND ENVIRONMENTAL APPLICATIONS

M.E. Arias[1], A. Blánquez[1], J. Rodríguez[1], M.E. Eugenio[2], R. Martín-Sampedro[2], M. Hernández[1]
[1]University of Acalá, Department of Biomedicine and Biotechnology, Alcalá de Henares, Spain
[2]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA-CIFOR, Departamento de Productos Forestales, Madrid, Spain

Backgrounds

Laccases (E.C. 1.10.3.2) are considered appropriated tools for biotechnological purposes. In our group, lacasse produced by *Streptomyces ipomoeae* CECT 3341 (SilA) have been fully characterized. The most remarkable features of this bacterial enzyme are the halotolerance and the activity and stability at alkaline pHs. Based on these characteristics the enzyme can be considered as an eco-friendly strategy to solve environmental and technological problems.

Objectives

The aim of this study was to show up the efficiency of SilA laccase for the following purposes:

i) decolourization and detoxification of textile azo-dyes; *ii*) degradation and detoxification of quinolone based antimicrobials and, *iii*) biobleaching of eucalyptus kraft pulp.

Methods

Recombinant SilA laccase was used in the presence or absence of phenolic compounds as mediators. The suitability of laccase-mediator systems (LMS) for decolourization of azo-dyes (Tartrazine and Orange II) and degradation of Ciprofloxacin and Norfloxacin was screened by HPLC-DAD. The detoxification assays were carried out using Algaltoxkit. In addition, the effect of SilA-acetosyringone system on the biobleaching of eucalyptus kraft pulp was studied measuring kappa number, viscosity and CIE l*a*b coordinates.

Conclusions

SilA-acetosyringone system produced azo-dyes decolourization and fluoroquinolones degradation with an 80-90 % efficiency. Moreover, this system was able to detoxify most of assayed pollutants although no direct correlation between degradation and detoxification was observed for Ciprofloxacin. Finally, biobleached pulps with LMS showed a kappa number decrease and a brightness increase without decreasing the viscosity values. CIE *L*a*b** color coordinates demonstrated that biobleached pulps presented the best optical properties even after an accelerated ageing process was applied.

FEMS7-1344
Biotechnology / Synthetic Biology / Systems Biology

MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES OF CLOSTRIDIUM BEIJERINCKII NCIMB 8052 DURING BATCH BUTANOL PRODUCTION

I. Paredes[1], K. Guerrero[1], J. Quintero[1], R. Gallardo[1], G. Aroca[1], J.C. Gentina[1], R. Conejeros[1]
[1]Pontificia Universidad Católica de Valparaíso, Escuela de Ingeniería Bioquímica, Valparaiso, Chile

Backgrounds

The main characteristic of solvent production by *Clostridium sp*. is that the fermentation typically proceeds in two phases, acids are formed first, followed by solvents production. Both acids and solvents present in the broth influence the physiological state of the population, and in the case of acids, it is believed that they contribute to the initiation of sporulation. However, these two phases have been only distinguished in *C. acetobutylicum*. In some cases, these phases are not clearly separable but currently these kinds of systems are not well studied. The relationship between transition from acidogenic to solventogenic and sporulation are still unclear.

Objectives

To assess the morphological and physiological changes of *Clostridium beijerinckii* NCIMB 8052 during batch ABE fermentation and to associate these changes with cell growth and acids and solvents production.

Methods

Clostridium beijerinckii was grown in a 2 L laboratory fermentor at 300 rpm, 37°C and controlled pH. Samples were analyzed by spectrophotometry for biomass, HPLC for dextrose and acids, and GC for butanol, ethanol and acetone. The morphological changes were followed by flow cytometry, staining and fluorescence microscopy.

Conclusions

The morphological and physiological changes of *C. beijerinckii* during batch cultivation are not synchronized for the whole population, but rather a heterogeneous distribution of morphologies can be found at different times. The staining methodology allowed to differentiate morphological forms of the *C. bejerinckii* cells during the fermentation. Due to the evident changes in the cell-wall during the evolution of the fermentation, different interaction with the Syto-9 and PI dyes were observed.

FEMS7-2153
Biotechnology / Synthetic Biology / Systems Biology

COMBINATORIAL BIOSYNTHESIS OF NATURAL AND NON-NATURAL PLANT-DERIVED PHENOLS IN MICROORGANISMS

J. Aschenbrenner[1], *J. Marienhagen*[1], *M. Bott*[1]
[1]*Forschungszentrum Jülich, Institute of Bio- and Geosciences IBG-1: Biotechnology, Jülich, Germany*

Backgrounds

The group of plant (poly)phenols comprises a larger number of secondary metabolites with a huge potential for applications in food and pharmaceutical industries (e.g. as flavours, colourants, therapeutic agents, antibiotics, etc.). Enzymes related to the phenylpropanoid metabolism are believed to have a rather broad substrate spectrum and thus might be able to accept a variety of different substrates.

Objectives

An *Escherichia coli* strain harbouring a synthetic pathway for phenylpropanoid synthesis will be constructed and evaluated for the possibility to convert chemically synthesized precursors to the respective monolignols. These alcohols will serve as building blocks for the (microbial) synthesis of larger compounds of pharmacological interest.

Methods

E. coli BL21 (DE3) was engineered for the plasmid-based expression of four genes encoding for four enzymes of the complete phenylpropanoid pathway starting from aromatic amino acids. The genes are organized as a synthetic operon under the control of an inducible T7-promoter. The constructed strain was then evaluated for the microbial monolignol production from various natural and non-natural precursors. Experiments were performed in shake flasks and the compounds were determined by HPLC and LC/MS.

Conclusions

Due to relaxed substrate specificity of studied enzymes of the synthetic monolignol pathway, which was successfully established in *E. coli*, this strain appears to be suitable for the microbial production of numerous natural and non-natural monolignols. The versatility of this single strain will provide access to the synthesis of more complex compounds such as flavonoids, stilbenes or lignans with interesting properties.

FEMS7-2802
Biotechnology / Synthetic Biology / Systems Biology

INJECTION OF NANOBODIES INTO TUMOR CELLS USING A SYNTHETIC INJECTOR E. COLI (SIEC) STRAIN

A. Asensio[1], *B. Álvarez*[1], *D. Ruano-Gallego*[2], *L.Á. Fernández*[1]
[1]*Centro Nacional de Biotecnología CNB-CSIC, Microbial Biotechnology, Madrid, Spain*
[2]*Imperial College, Life Sciences, London, United Kingdom*

Backgrounds

Type 3 secretion systems (T3SS) assemble supramolecular needle-like protein complexes, called injectisomes, able to translocate protein effectors into host cells. One attractive application of T3SS is the injection of therapeutic proteins, such as antibodies, into human cells (e.g. tumor cells). To avoid the use of pathogenic strains carrying T3SS, we engineered the commensal *Escherichia coli* K-12 strain to express injectisomes from the enteropathogenic *E. coli* (EPEC) strain. The resulting strain, named Synthetic Injector *E. coli* (SIEC), was able to translocate EPEC effectors (e.g. Tir) into human tumor cells.

Objectives

The main objective is to develop an expression system that allows the efficient translocation of single domain antibody fragments (nanobodies) into tumor cells using SIEC bacteria.

Methods

We assessed the controlled expression in SIEC of nanobodies fused to various T3-secretion signals and in the presence of different T3-chaperones. We evaluated and quantified the translocation levels of these gene constructs into tumor cell lines using β-lactamase as a reporter, both from high-copy plasmid vectors and single-copy gene integrations in SIEC chromosome. These experiments allowed us to select an optimal promoter, T3-signal and chaperone for controlled and efficient nanobody translocation.

Conclusions

We engineered SIEC strain to deliver nanobodies into the cytosol of tumor cells in a controlled manner both from plasmid and single-copy chromosomal expression. The developed system could be also of application for other therapeutic proteins.

FEMS7-0740
Biotechnology / Synthetic Biology / Systems Biology

BACTERIOPHAGE FUNCTIONALIZED POLYPYRROLE FOR DETECTION OF CLOFIBRIC ACID USING A MICROBALANCE SENSOR SYSTEM

I.H. Baek[1,2], V. Helms[2], Y. Kim[1]
[1]Kist europe, Environment safety group, Saarbrücken, Germany
[2]Saarland University, Center for Bioinformatics, Saarbrücken, Germany

Backgrounds

Molecularly imprinted polymer (MIP) is a central part of chemical removals or biosensors that depend on functionalized noncovalent recognition cavities. The recognition elements in biosensors usually consist of antibodies, enzymes or other biological receptors that are immobilized on the sensor surface. Filamentous bacteriophages are a member of the family *Inoviridae.* These are various used for material science, drug delivery, tissue engineering, energy and biosensor. Genetically modified bacteriophages can give to various binding recognition cavities including ionic interactions. clofibric acid (CA) is one of the metabolite form of fibrate drugs. These are commonly used in prescription drugs to treat diseases related to the lipid lowering symptom in the blood of both human and domestic animals.

Objectives

Multi-functionalized molecularly imprinted polymer, using genetically engineered filamentous bacteriophage was used for detection of acidic pharmaceutics (CA)

Methods

E. coli were used for plasmid and phage amplification respectively. Phage preparation was performed by PEG/NaCl precipitation. Cyclic voltammetry were performed using an electrochemical flow module of QCM-D, which consists of a working electrode.

Conclusions

A novel MIP combined with filamentous bacteriophages was shown to bind specifically to the substrate CA due to specific noncovalent interactions. Also, we described the use of functionalized phage pyrrole MIP for the real time monitoring of CA. The functionalized phage conductive polymer can be used for efficient on/off sensing of targets. The new method appears to be a promising unique and versatile method with high and efficient reproducibility that should have many applications in the field of sensors, electronics as well as biomedical engineering.

FEMS7-3222
Biotechnology / Synthetic Biology / Systems Biology

HYDROGEN OVERPRODUCING NITROGENASES OBTAINED BY RANDOM MUTAGENESIS AND HIGH-THROUGHPUT SCREENING

E. Barahona[1], E. Jiménez-Vicente[2], I. Álvarez- Melcón[1], L.M. Rubio[1]
[1]Centro de Biotecnología y Genómica de Plantas. Universidad Politécnica de Madrid UPM - Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA. Campus Montegancedo UPM. 282 23-Pozuelo de Alarcón Madrid- Spain., Biotecnología, Pozuelo de Alarcón, Spain
[2]Virginia Tech, Biochemistry, Blacksburg, USA

Backgrounds

The great interest for hydrogen (H_2) as energy vector underlies research programs aimed to improving its sustainable biological production. During the process of biological nitrogen fixation the enzyme nitrogenase generates a minimum of one molecule of H_2 per turnover. We present a method for the high-throughput selection of nitrogenase variants with enhanced H_2 production. A *Rhodobacter capsulatus* strain has been re-engineered to generate a fluorescent signal in response to nitrogenase-produced H_2. A combination of random mutagenesis and fluorescence-activated cell sorting (FACS) is then used to select H_2-overproducing mutants of *R. capsulatus*.

Objectives

To develop optimized biocatalysts for hydrogen production and to design a biotechnological tool for the detection of H_2-overproducing bacteria.

Methods

The biotechnological tool consists of a library of (10^5-10^6) random nitrogenase variants and a biological H_2 sensor, which is based on the sensing hydrogenase of *R. capsulatus* and in the engineered state produces fluorescent signals. High-throughput selection was achieved by coupling this tool to FACS cytometry.

Conclusions

We have re-engineered the natural H_2 sensing system of *R. capsulatus* and combined it with FACS for the high-throughput selection of nitrogenase variants with enhanced H_2 production that might independently retain their N_2 fixation activity. This technology possesses great potential to identify nitrogenase amino acid substitutions leading to H_2-overproducing variants that could be mimicked in nitrogenases from other microorganisms, expanding the impact of the findings. This tool was also used to perform a genome-wide screening of mutations leading to enhanced H_2 production in *R. capsulatus*.

FEMS7-2915
Biotechnology / Synthetic Biology / Systems Biology

MOROCCAN BIOACTIVE ACTINOBACTERIA ISOLATES PRODUCING EFFLUX PUMPS INHIBITORS OF RESISTANT BACTERIA

A. Azmani[1], A. Nafis[1], L. Hassani[1], J. Wink[2], M. Barakate[1]
[1]Laboratory of Biology and Biotechnlogy of Microorganisms .Department of Biology, Faculty of Sciences Semlalia Cadi Ayyad University. P.Box.: 2390 Marrakech 40000, Morocco
[2]Helmholtz Center for infection research. Microbial Agents (MWIS) Research Group leader Microbial Strain Collection (MISG) Braunschweig.Germany

Background:

Traditional antimicrobials antibiotics are increasingly suffering from the emergence of multidrug resistance among pathogenic microorganisms. Among the antibiotic resistance mechanisms, efflux pumps have recently received a particular attention. Hence, there is an acute need for new active agents.

Objectives:

Reducing the rate of emergence of antibiotic-resistant is our objective by studying the ability of Moroccan actinobacteria isolates to produce natural efflux pumps inhibitors (EPI) of medical interest.

Methods:

210 actinobacteria isolates were screened for their ability to produce efflux pumps inhibitors using agar diffusion method. As test strains we have used in this study the wild type strain *Escherichia coli* AG100 and its mutant AG100A with non- functional pump efflux system and the two clinical strains of *Staphylococcus.aureus*: the sensitive one SA-1199 and its mutant SA1199B. Phe-Arg-β-naphtylamide (PAβN) which inhibits the efflux system of many bacteria was used as control. The extraction and purification of bioactive compound produced by the most promising isolate were conducted from large scale fermentation on the Bennett medium after extraction by ethyl acetate and purification by HPLC. The structure elucidation was determined after spectroscopic and spectrometric analyses. Taxonomic study to genus and species level of the producing isolate was conducted using morphological, physiological, chemotaxonomic and molecular studies.

Results:

Our screening program showed that among the 210 screened actinobacteria isolates, 9% were able to produce EPI with 7% and 3% respectively against Gram negative and Gram-positive bacteria. The highest EPI activity was produced by the strain Z332 which produce a derivative new compound. This strain could be a new strain of the genus *Streptomyces* as it is most closely related to *S. bellus* NBRC 12844 and *S. coerulescens* CSSP046 species on the basis of 16rDNA sequence while significant differences were obtained on the comparison of the morphological and physiological characteristics of the strain with those of the two nearest species.

Conclusion

Screening only a subsection of our natural product library led to purify a specific EPI capable of sensitizing the Gram-negative bacteria to antibiotics to which they are ordinarily intrinsically resistant. This result demonstrates the great potential of this approach in expanding antibiotic effectiveness regarding the growing challenge of resistance.

FEMS7-0703
Biotechnology / Synthetic Biology / Systems Biology

MURAMIDASE ACTVITY IN A A 110-KDA MEMBRANE PROTEIN PRODUCED FOR PEDIOCOCCUS ACIDILACTICI ATCC 8042

P.A. Barbosa-González[1], I. García-Cano[1], A. Farrés[1]
[1]Universidad Nacional Autónoma de México, Departamento de Alimentos y Biotecnología, Mexico City, Mexico

Backgrounds

Pediococcus acidilactici ATCC 8042 has been used as starter culture in meat products and exerts a biopreservative effect. This working group hads reported this strain produced a 110- kDa protein with antibacterial activity (García Cano *et al*., 2011). Its gene was sequenced and proved to have homology with a protein with unknown function which contained dominions both for a lytic phage protein and an ABC transporter.

Objectives

The antimicrobial effect of 110-kDa protein represents an enigma because its sequence is not similar to known antibacterials. This research intends to gain insight into its mode of action

Methods

To analyze the individual antimicrobial activity of the 110-kDa protein the gene for its expression was cloned using the vector pET19 and *E. coli* BL 21 as a host, resulting in a recombinant protein with observable antimicrobial activity against important pathogens (*S. aureus, L. monocytogenes, S. pyogenes, E.coli, and S. typhimurium*) . The activity was detected both in zymograms and in growth inhibition tests. Enzymatic activities that may have an effect on cell wall lysis were assayed with spectrophotometric methods and synthetic substrates and the 110-kDa recombinant protein showed muramidase activity. This would not be a canonical muramidase as it does not present homology to any reported peptidoglycan hydrolase. More details into its action mechanism need to be analyzed, as its probably effect on the permeability of bacteria.

Conclusions

The 110-kDa membrane protein is a muramidase and it may be used as an option to reduce and avoid microbial spoilage and contamination

FEMS7-2452
Biotechnology / Synthetic Biology / Systems Biology

PROWOOD: PROTECTION OF WOOD BY NOVEL BIO-BASED SOLUTIONS

K. Kosalková[1], A. Sola-Landa[2], L. García-Calvo[1], R. Pérez-Redondo[2], C. Sánchez-Orejas[3], M. Calonge[3], C. Barreiro[4,5]
[1]INBIOTEC Instituto de Biotecnología de León, Fungal Biotechnology, Leon, Spain
[2]INBIOTEC Instituto de Biotecnología de León, Actinobacteria, Leon, Spain
[3]INBIOTEC Instituto de Biotecnología de León, Toxicology, Leon, Spain
[4]INBIOTEC Instituto de Biotecnología de León, Proteomics, Leon, Spain
[5]Universidad de León, Área Microbiología. Biología Molecular., León, Spain

Backgrounds

The European Union (EU27) woodworking industry consists today of more than 380,000 companies and employs more than 2.1 million workers. The European industrial interest on wood decay processes is both to accelerate (biorefinery) and to prevent wood degradation (decay). While the hydrolytic degradation processes are extensively studied and improved for biofuels production, the prevention of wood degradation has received less attention, even though, the preservation of wood structures against decomposition is an old challenge.

Objectives

New protective bio-coatings are the core of the ProWood Consortium in order to develop bio-coating solutions based on three alternate sources of novel eco-friendly anti-wood decay agents all combined with three novel, specially developed, coatings.

Methods

Methods for isolation and characterization of anti-microbial or antagonist compounds, as well as, microorganism involved in metal corrosion (biofouling) will be transferred to wood decay characterization.

Conclusions

ProWood is a well-balanced interdisciplinary cluster of outstanding molecular biologists, (bio)chemical engineers joined to economic and social-relevant industrial partners selected for their know-how in biotechnology, bio-coating development, wood handling and validation processes. The Consortium is resulting in innovative bio-coatings to reach an industrially demanded product to cover societal needs in wood protection in a less polluting manner.

FEMS7-2484
Biotechnology / Synthetic Biology / Systems Biology

BIOTYPES ANALYSIS OF CORYNEBACTERIUM GLUTAMICUM DEMONSTRATES THE EXISTENCE OF INTRA-SPECIES VARIATIONS

F. Pérez-García[1], M.F. Vasco-Cárdenas[1,2], A. del Árbol[1], A.M. Ibañez[1], C. Barreiro[1,2]
[1]INBIOTEC Instituto de Biotecnología de León, Proteomics, Leon, Spain
[2]Universidad de León, Área de Microbología. Biología Molecular., León, Spain

Backgrounds

Mutagenesis with chemical methods, irradiation or genetic manipulation have been the traditional ways to enhance the yield of industrial microbial products or improve relevant strains. Insufficient studies have been developed on final product resistance improvement and even fewer on the naturally-generated biotypes, which could decrease the artificial mutagenesis ratios. This is a capital fact in the case of GRAS microorganisms involved in food, feed and cosmetics production as *Corynebacterium glutamicum*.

Objectives

The analysis of four biotypes of *C. glutamicum* involved in the dicarboxylic acid production are the core of the presented work. Specifically, the proteome analysis of these biotypes growing in an industrial relevant amount of itaconic acid are the proof of concept.

Methods

Cells were cultured in the defined medium CGXII with and without itaconic aid. 2D-DIGE analysis of the intra-cellular proteome were performed and proteins were subsequently identified by means of a MALDI-TOF/TOF mass spectrophotometer. Metabolic pathway analysis was carried out by using the BioCyc web server, which allowed the identification of pathways involved in the itaconic acid resistance among the biotypes.

Conclusions

The relevance of a preliminary biotype screening is demonstrated along the present analysis as a platform for strain production improvement using a proper selection of resistant biotypes facing metabolite toxicity. Besides, an analysed biotype, which presents a more active central metabolism, an optimized nutrient uptake and better protection against osmotic stress and acidification, is presented an excellent candidate for itaconic acid production.

FEMS7-1990
Biotechnology / Synthetic Biology / Systems Biology

DISCOVERY AND ENGINEERING OF NOVEL DEFENSIN-LIKE ANTIMICROBIAL PEPTIDES TO COMBAT FOOD SPOILAGE

M. Beckett[1], J. Kraszewska[1], T. James[1], U. Bond[1]
[1]Trinity College Dublin, Microbiology, Dublin, Ireland

Backgrounds

Antimicrobial peptides offer potential as novel therapeutics to combat food-spoilage bacteria. Our previous studies identified the peptide human beta-defensin 3, HBD3, as a potent antimicrobial agent for a wide range of food-spoiling bacteria. Thus, HBD3 is an excellent candidate to prevent food spoilage.

Objectives

To identify other peptides with antimicrobial activity similar to HBD3 and engineer peptides with increased activity.

Methods

We carried out an *in silico* screen using HBD3 as the comparative molecule, focusing on peptides of plant origin. We compared the antimicrobial activity of nine defensin-like peptides to the activity of HBD3, against the food-spoiling bacterium *Lactobacillus brevis*. Two of the peptides, Fabatin-2 and Cp-Thionin-2, displayed antimicrobial activity in the same concentration range as HBD3 under high ionic strength conditions. Several of the other peptides displayed antimicrobial activity under low ionic strength conditions. Further analysis revealed a direct correlation between activity and net positive charge of the peptide. A rational design approach was undertaken to increase the antimicrobial activity of a “low activity” peptide, Mungbean defensin PDF-1. First the structure of PDF-1 was compared to the tertiary structure of active peptides. We identified 3 regions of negative charge which were altered to increase the cationic charge of the peptide.

Conclusions

Subsequent testing showed this analogue to have greater activity than the oringianl PDF-1 peptide and any of the previously identified “high activity”peptides. Thus rational design, based on structural and physiochemical properties of short peptides can be used to engineer antimicrobial peptides which are effective at controlling food spoilage bacteria.

FEMS7-0286
Biotechnology / Synthetic Biology / Systems Biology

NEW ASPECTS OF REGULATION OF AN ANGUCYCLINE ANTIBIOTIC AURICIN IN STREPTOMYCES AUREOFACIENS CCM 34239

C. Bekeova[1], L. Feckova[1], R. Novakova[1], E. Mingyar[1], D. Homerova[1], J. Kormanec[1]
[1]Institute of Molecular Biology Slovak Academy of Sciences, Gene expression, Bratislava, Slovak Republic

Backgrounds

Streptomycetes are characterised by a complex morphological differentiation and production of secondary metabolites, including the majority of known antibiotics. The biosynthesis of secondary metabolites is strictly regulated, involving at least two different levels: a lower pathway-specific level using cluster-situated regulators and a higher one involving various global regulators [1]. We previously identified a gene cluster *aur1* in *Streptomyces aureofaciens* CCM 3239 responsible for production of a unique angucycline antibiotic auricin. Its biosynthesis is regulated by at least four pathway-specific regulators, including response regulator homologue Aur1P, TetR family regulator Aur1R, and two SARP family regulators Aur1PR3 and Aur1PR4 [2].

Objectives

Characterization of new putative regulatory gene *aur1O* and its involvement in regulation of auricin biosynthesis.

Methods

Growth of *S. aureofaciens* CCM3239 and analysis of auricin production was done as described in [3]. Disruption of the genes in *S. aureofaciens* CCM3239 was done as described in [3]. Bacterial two hybrid system was done as described in [4].

Conclusions

1, Deletion of the *aur1O* gene affected production of auricin, indicating its role in regulation of its biosynthesis.

2, The auricin biosynthetic promoter *aur1Ap* had decreased activity in the absence of *aur1O* gene, indicating the role of Aur1O in its activation.

3, Aur1O has been found to interact with the auricin pathway-specific regulator Aur1P, indicating the role of Aur1O as an co-activator of the *aur1Ap* promoter.

Acknowledgements. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0410 and by VEGA grant 2/0002/16 from Slovak Academy of Sciences.

References.

1. Bibb MJ (2005) *Curr Opin Microbiol* 8, 208-215

2. Kormanec J, Novakova R, Mingyar E, Feckova L (2014) *Appl. Microbiol. Biotechnol.* 98, 45-60.

3. Novakova R, Rehakova A, Kutas P, Feckova L, Kormanec J (2011) *Microbiology-SGM* 157**,** 1629-1639.

4. Sevcikova B, Rezuchova B, Homerova D, Kormanec J (2010) *J. Bacteriol*.192, 5674-5681.

FEMS7-3016
Biotechnology / Synthetic Biology / Systems Biology

SCREENING OF FEEDING STRATEGIES BASED ON TRIGGER EVENTS IN A 24-MICRO BIOREACTOR PLATFORM

C. Bernal Martinez[1], *C. Santos Fernandez*[1]
[1]*Applikon Biotechnology BV, Product Management, Delft, The Netherlands*

Backgrounds

Several bioprocesses exploit feeding strategies such as the minimization of acetate production in microbial cell culture (Johnson *et al*., 2002, Kim *et a*l., 2004, Andersen *et al.*, 2001). Some of these feeding strategies use values for pH or dissolved oxygen (dO2) as a trigger point.

.

Objectives

The objetive was to test the event (trigger point) based control using micro-scale fermentation. The availability of a platform able to perform this approach on a micro-scale cultivation is essential for strain optimization strategy.

Methods

The micro-Matrix has been used to run 24 fermentations using *E.coli (*Chen *et al*., 1997). Applikon's micro-Matrix product is a platform that holds 24 micro bioreactors (working volume 2-5 mL) with individual control of pH, dO2 and temperature. The micro-Matrix is able to program event-based control whereby the control strategy is based on a triggered event

Conclusions

In the present work, two different feeding strategies in an *E.coli* cultivation have been applied based on i) pH above 7.1 and ii) dO2 above 50%, using the work of Chen *et al*., 1997 as a reference. The results demonstrate that the micro-Matrix can be successfully used for the screening of different feeding strategies.

FEMS7-2727
Biotechnology / Synthetic Biology / Systems Biology

ASSESSMENT OF ANTIFUNGAL AND ANTI-AFLATOXIGENIC EFFECT OF PROBIOTICS: A NOVEL STRATEGIES FOR REDUCING MYCOTOXIN LEVELS IN PEANUTS

S. Bhutada[1]
[1]*Sanjivani Arts - Commerce and Science College, Department of Microbiology, Ahmednagar, India*

Backgrounds

Contamination of peanuts with mycotoxins, particularly aflatoxins, is a worldwide problem that affects both food safety and agricultural economies. Aflatoxins are carcinogenic mycotoxin, produced by *Aspergillus* species. These molds infect food crops in warm humid conditions causing economic losses and affecting the consumers' health adversely.

Objectives

Due to the ubiquitous occurrence of aflatoxins, preventive and remediative measures are necessary including detoxification techniques. The objectives of the present study are to determine the antifungal and anti-aflatoxigenic activity of probiotics for reducing the mycotoxin levels in Peanuts.

Methods

In this study, antifungal activity and Anti-aflatoxigenic effect of Nine probiotic strains against *Aspergillus flavus* and *Aspergillus parasiticus* were studied. The aflatoxin secreted was analyzed and quantified by both UV spectrophotometer and HPLC.

Conclusions

It was found that *Lactobacillus plantarum* showed antifungal (65.89% reduction) and anti-aflatoxigenic (94.56% reduction) activity against *A. flavus* whereas *A. parasiticus* was inhibited by *Lactobacillus plantarum* with the antifungal reduction of 64.75% and anti-aflatoxigenic reduction of 91.32%. The research outcome concluded that *Lactobacillus plantarum* may be used as potential probiotic for reducing the aflatoxin content in Peanuts. Use of Probiotics should be encouraged for use as a bio-detoxification agent for aflatoxins.

FEMS7-1662
Biotechnology / Synthetic Biology / Systems Biology

UNDERSTANDING COXIELLA VACUOLE BIOGENESIS USING MULTI-PARAMETRIC HIGH-CONTENT SCREENING

M. Bonazzi[1], K. Lambou[1]
[1]Centre d'études d'agents Pathogènes et Biotechnologies pour la Santé, Cell Biology of Bacterial Infections, Montpellier, France

Background

Coxiella burnetii is an obligate intracellular bacterium responsible of severe outbreaks of the zoonosis Q fever. The primary targets of *Coxiella* are alveolar macrophages, however bacteria can invade phagocytic and non-phagocytic cells and disseminate to other tissues and organs, such as the liver and heart, giving rise to hepatitis and endocarditis. Due to its remarkable infectivity and its environmental stability, *Coxiella* is considered a category B biothreat. Upon internalisation by both phagocytic and non-phagocytic cells, bacteria remain confined within *Coxiella*-containing vacuoles (CCVs), tight fitting compartments that mature along the endocytic pathway. Key to the successful colonisation of host cells is the translocation of bacterial effectors translocation by a Dot/Icm type IVb secretion system.

Objectives

Bioinformatics analysis identified over 300 *Coxiella* candidate effectors, a third of which have been validated for secretion. Many *Coxiella* effectors remain completely uncharacterised, it is clear however that several of these are encoded by eukaryotic-like genes (EUGENs), suggesting an interaction with host cell proteins and pathways.

Methods

Using transposon mutagenesis coupled to multi-parametric high-content screening, we have investigated the role of Dot/Icm core protein during *Coxiella* infections, defined the impact of known effectors in different steps of the infectious cycle and identified novel candidate effectors.

Conclusions

Coxiella Vacuolar Proteins (Cvps) seem to be directly implicated in vacuole biogenesis via the manipulation of host vesicular trafficking, allowing CCVs to intercept and recruit membranes, proteins and lipids from the endocytic, autophagy and recycling pathways. We have recently reported that CvpB associates to CCVs by interacting with host phosphoinositides and modulates PI(3)P levels to facilitate homotypic fusion between CCVs. Transposon insertions in *cvpB* lead to a multivacuolar phenotype, *Coxiella* replicating in multiple, isolated CCVs. Interestingly, despite the intracellular replication of *cvpB* mutants remain unaffected, defective CCVs development as *in vivo* relevance as demonstrated using the insect model *Galleria mellonella*.

FEMS7-3035
Biotechnology / Synthetic Biology / Systems Biology

USE OF RECOMBINANT MULTIEPITOPE PROTEINS AS A STRATEGY TO AID IN THE DIAGNOSIS OF CRYPTOCOCCOSIS AND IN THE IDENTIFICATION OF ENDEMIC AREAS

R. Brandão[1], A. Faria[2], H. Andrade[2], L. Martins[1], A. Silva[1], S. Monte[1]
[1]Federal University of Piaui, Laboratório de Imunogenética e Biologia Molecular LIB, Teresina, Brazil
[2]Federal University of Minas Gerais, Departament of Parasitolgy, Belo Horizonte, Brazil

Backgrounds

Cryptococcosis is a worldwide life-threatening systemic disease caused by *Cryptococcus neoformans* (Genotype VNI-VNIV, VNV) and *Cryptococcus gattii* (Genotype VGI-VGIV). Despite major advances in the search for a rapid test with the goal of an early diagnosis, estimations are that almost one million cases of cryptococcal meningitis in HIV-infected subjects occur annually, causing more than 600,000 deaths. This shows that early diagnosis of cryptococcosis remains a challenge that science and the health system need to address. As important as the early diagnosis is the identification of endemic areas and control of the fungus spreading.

Objectives

To test recombinant multiepitope proteins for the development of a rapid test or epidemiological tool aiming at the identification of endemic regions of cryptococcosis.

Methods

Previously, proteomics and bioinformatics methods were combined and potential antigenic targets were recognized by anti-Cryptococcus antibodies. Here, it was designed and synthesized 4 recombinant multiepitope proteins (A, B, C and D), constructed with the combination of potential peptides, and crossed them with sera from cryptococcosis patients and controls.

Conclusions

The recombinant multiepitope proteins A, B and C showed sensitivity between 57.14 to 88.57%, specificity of 90% and AUC between 0.71 and 0.91. The chimeric D protein presented better results, with sensitivity of 88.57%, specificity of 100% and AUC of 0.97. The results suggest that the recombinant multiepitope proteins in combination with lateral flow assay may increase the ability in diagnosing patients with cryptococcosis and may be useful as an epidemiological tool to identify endemic areas.

FEMS7-2523
Biotechnology / Synthetic Biology / Systems Biology

ALTERING THE SACCHAROMYCES CEREVISIAE POLY(A) BINDING PROTEIN (PAB1) FOR LEVERAGING CELLULAR ROBUSTNESS

P. Branduardi[1], *M. Brambilla*[1], *F. Martani*[1], *A. Mento*[1], *S. Bertacchi*[1], *D. Porro*[1]
[1]*University of Milano Bicocca, Department of Biotechnology and Biosciences, Milano, Italy*

Backgrounds

When exploited as cell factories, microbial cells are exposed to harsh conditions impairing titer, yield and productivity of the fermentative processes. The development of robust strains therefore represents a pivotal but not easy task to accomplish for the implementation of cost-effective bioprocesses.

Objectives

In our laboratory, we explored the possibility to leverage *S. cerevisiae* potential in terms of cellular robustness through the modulation of a “hub” element involved in post-transcription events. In fact, although transcription shapes the adaptive response to stress, the mechanisms regulating the fate of newly synthesized mRNAs are crucial for tuning the final effect of eukaryotic gene expression.

Methods

The poly(A) binding protein Pab1, as stress granules component, was chosen as the target for obtaining widespread alterations in mRNA metabolism and for selecting stress tolerant phenotypes. Here we will show how either the modulation of Pab1 levels as well as the selection of specific variants through a screening protocol allowed to retrieve strains ameliorated against different stressors; these strains demonstrated better performances under industrially relevant conditions, when compared with controls. Furthermore, the construction of Pab1 chimeras is offering the possibility to further study the contribution of the different parts of the protein in the assembly and clearance of stress granules, with consequences on cellular robustness.

Conclusions

These findings pave the way for a novel approach to unlock industrially promising phenotypes through the modulation and/or the synthetic reconstruction of a post-transcriptional regulatory element.

FEMS7-1386
Biotechnology / Synthetic Biology / Systems Biology

RELATIONSHIP BETWEEN SPORULATION AND SOLVENT PRODUCTION IN CLOSTRIDIUM BEIJERINCKII NRRL B-598

B. Branská[1], J. Kolek[1], M. Drahokoupil[1], M. Vasylkivska[1], P. Patakova[1]
[1]University of Chemistry and Technology - Prague, Department of Biotechnolohy, Prague 6, Czech Republic

Backgrounds

Although industrial bio-solvent production using genus *Clostridium* had 100 year anniversary last year, it is still in the spotlight, with many not fully revealed mechanisms and regulations. One of such uncovered mechanism is a relationship between sporulation and solvent production. In *C. acetobutylicum,* the most studied representative of solventogenic Clostridia, sporulation and the onset of solvents formation seems to be directly interconnected, however this may not apply for other industrially relevant Clostridia.

Objectives

The objective of this study was to find relations between production parameters and particular stages of sporulation cycle in *C. beijerinckii* NRRL B-598 (formerly *C. pasteurianum* NRRL B-598).

Methods

Spores formation together with solvent production and culture viability were monitored during a whole production and sporulation cycle under specific conditions (supporting/repressing sporulation). A high throughput flow cytometric method based on a combination of light scatter and fluorescence characteristics was employed for spores, live and dead cells enumeration.

Conclusions

Morphological changes typical for spores formation could be observed at the onset of solvent formation, similarly to *C. acetobutylicum*. However, it was proved that it does not apply vice versa, meaning that for *C. beijerinckii* NRRL B-598 solvent production can be undisturbed when sporulation is limited or is not observed at all.

Acknowledgement

This work has been supported by grant project GACR 17-00551S.

FEMS7-0775
Biotechnology / Synthetic Biology / Systems Biology

FROM GREENHOUSE GASES TO BIOFUEL: CARBON CAPTURE TO N-BUTANOL BY CLOSTRIDIUM BEIJERINCKII

W.J. Sandoval-Espinola[1], M. Chinn[2], M. Thon[3], J.M. Bruno-Barcena[1]
[1]North Carolina State University, Plant and Microbial Biology, Raleigh, USA
[2]North Carolina State University, Agricultural and Biological Engineering, Raleigh, USA
[3]Universidad de Salamanca, Department of Microbiology and Genetics, Villamayor, Spain

Backgrounds

Recent efforts to combat increasing greenhouse gases emissions include their capture into advanced biofuels, such as butanol. However, butanol research has traditionally been centered solely on its generation from sugars, because solventogenic *Clostridiums*, such as *C. beijerinckii* are considered heterotrophic organisms.

Objectives

To monitor in-line the endogenous fermentation gases by *Clostridium beijerinckii* and to assess its capability to assimilate syngas (CO + CO2 + H2) into fermentation products.

Methods

We performed chemostat (D = 0.135 h-1) fermentations while continuously flowing syngas (CO + CO2 + H2) at increasing steady-state concentrations, while analyzing mass balances. We also performed batch experiments with C-13 labeled CO2 to confirm its assimilation into products. We further performed genome- and transcriptome-wide analysis, focusing on autotrophy-related genes.

Conclusions

Our results show the mixotrophic CO_2 and H_2 re-assimilation by n-butanol-producer *C. beijerinckii*. This was detected as synchronous CO_2/H_2 oscillations by direct (real-time) monitoring of the fermentation gasses. Additional functional analysis demonstrated syngas (H_2, CO_2 and CO) assimilation, increasing total carbon recovery above typical heterotrophic values. This was also confirmed by C-13-labeled CO_2 recovered into products. Further genome- and transcriptome-wide analysis, revealed the transcription of key Wood-Ljungdahl pathway and other autotrophy-related genes. Therefore, this report provides genetic and physiological evidence of inorganic carbon-capture by *C. beijerinckii.*

FEMS7-2285
Biotechnology / Synthetic Biology / Systems Biology

ENGINEERING OF SUGAR TRANSPORT CAPABILITIES OF CORYNEBACTERIUM GLUTAMICUM FOR IMPROVED GROWTH ON D-XYLOSE

C. Brüsseler[1], A. Radek[1], K. Krumbach[1], J. Gätgens[1], T. Polen[1], N. Tenhaef[1], S. Noack[1], J. Marienhagen[1]
[1]Forschungszentrum Juelich GmbH, IBG-1, Juelich, Germany

Backgrounds

Naturally, *Corynebacterium glutamicum* cannot utilize the D-xylose. We have successfully engineered this organism to solely grow on this pentose by functional integration of the Weimberg pathway originally discovered in *Caulobacter crescentus*.

Objectives

Improvement of growth of *C. glutamicum* in D-xylose-containing defined medium.

Methods

C. glutamicum was subjected to an adaptive laboratory evolution (ALE) experiment for improved growth on D-xylose-containing defined medium. Resulting strains were characterized in cultivations in the shake-flask and microtiter plate format and additional genome sequenced. An LC-MS-based method for the quantification of D-xylose was developed and used for the detailed characterization of selected strains with regard to D-xylose consumption.

Conclusions

The ALE yielded several strain variants showing improved growth on D-xylose-containing medium. Conducted genome sequencing helped to identify a frame shift mutation in the gene encoding a transcriptional repressor. Through deletion of this repressor we were able to verify this effect on overall cell-growth in the wild-type background. In addition, we could functionally integrate all genes of the Weimberg pathway into the chromosome of this *C. glutamicum* strain. To our best knowledge this is the first plasmid-free *C. glutamicum* strain being able to grow solely on D-xylose employing the Weimberg pathway.

FEMS7-2012
Biotechnology / Synthetic Biology / Systems Biology

CHIMERIC LEADERS: TOOLS OF SYNTHETIC BIOLOGY FOR DIFFERENT DIRECTED PEPTIDE MODIFICATIONS

A. Buivydas[1], S.W. Fuchs[2], J. Piel[2], O.P. Kuipers[1]
[1]University of Groningen, Molecular Genetics, Groningen, The Netherlands
[2]ETH Zürich- Institute of Microbiology, Department of Biology, Zürich, Switzerland

Backgrounds

Posttranslationally modified antimicrobial peptides exhibit a potent antimicrobial activity, stability and high target specificity. Some of the peptides are more active even than some antibiotics. All the characteristics are endorsed by posttranslational modifications introduced during biosynthesis steps by dedicated enzymes. Maturation of a peptide begins with a propeptide which is guided by specific modification events due to a leader peptide (an amino acid sequence recognized by particular modification biomodule). The leader peptide is proteolitically removed in the last steps of peptide maturation rendering the modified peptide active.

Objectives

To design plug-and-play biomodules for production of novel peptides containing simultaneous posttranslational modifications of different origins.

Methods

We used synthetic biology approaches to design and implement biomodules for biosynthesis of a peptide with combinatorial modifications of choice. Codon-optimized synthetic genes of designed model peptides to be modified were employed together with dedicated modification machineries.

Conclusions

This is a unique case where posttranslational modifications from two different peptide classes are fused together into a single peptide chain *in vivo*. Here we also show the possibilities of employing chimeric leader peptides for substrate recognition and processing by particular posttranslational modification machineries.

FEMS7-2655
Biotechnology / Synthetic Biology / Systems Biology

COMPOSTING OF SEWAGE SLUDGE WITH SEMIPERMEABLE FILM TECHNOLOGY: MICROBIAL DIVERSITY AND ENZYMATIC ACTIVITIES THROUGHOUT THE STAGES OF THE PROCESS

C. Calvo[1], T. Robledo-Mahón[1], A. Rodríguez-Calvo[1], M. Manzanera[1], J. González-López[1], E. Aranda[1]
[1]Universidad de Granada, Microbiology- Institute of Water Research, Granada, Spain

Backgrounds

Composting is a bio-active process in which a large number of microorganisms are involved. Among these microorganisms, fungi and bacteria have diverse enzymatic activities that help to the stabilization of the organic matter and allow obtaining the compost, a final product which is safe and useful as an agricultural soil amendment. The starting products and the operational conditions have a high influence in the microbial diversity and will determine the process characteristics

Objectives

The main objectives were to investigate, in a full-scale real composting pile, the evolution of the diversity of bacteria and fungi during the composting process and to correlate the predominant groups and the enzymatic activities of the pile

Methods

The pile was built in real-scale mixing sewage sludge with vegetal pruning wastes as bulking agent. The composting phase was performed under Gore-Tex® semipermeable cover and the maturation phase was developed in an opened windrow system. Analysis of bacterial and fungi communities was performed by Illumina Miseq sequencing. Enzymatic activities: dehydrogenase, protease, phosphatase, β-glucosidase and arylsulphatase activities were determined at each composting phase

Conclusions

Actinomycetales and *Bacilalles* (bacteria) and *Eurotiales* and *Sordariales* (fungi) were the major taxa at the beginning of composting and during the maduration stage, while the highest diversity was detected between 15 and 30 days of composting. Protease, dehydrogenase, β-glucosidase and arylsulfatase activities were higher during the composting phase under Gore-Tex® cover, whereas acid and alkaline phosphatase activities decreased in the maturation stage.

Acknowledgement: Project funded by Junta de Andalucía (RNM-7370)

FEMS7-1493
Biotechnology / Synthetic Biology / Systems Biology

CELLULASE-PRODUCING BACTERIA ISOLATED FROM PERUVIAN SALTERNS USING SUGAR BEET PULP AS SUBSTRATE

C.N. Flores-Fernández[1], A.I. Zavaleta[1], P.E. Canales[1], M. Cárdenas-Fernández[2], J.M. Ward[2], G.J. Lye[2]
[1]Laboratorio de Biología Molecular - Facultad de Farmacia y Bioquímica, Universidad Nacional Mayor de San Marcos, Lima, Peru
[2]Deparment of Biochemical Engineering, University College London, London, United Kingdom

Backgrounds

Sugar beet is an important crop for sugar production in various parts of the world, and the pulp is the main by-product from sugar processing that contains high concentration of cellulose (25%). Halophilic bacteria are recognized for simple nutritional requirements and activity under harsh conditions.

Objectives

To isolate cellulase-producing bacteria from Peruvian salterns using sugar beet pulp as substrate.

Methods

Soil samples were collected from Peruvian salterns and cultured in a medium supplemented with 0.2% yeast extract, 5% NaCl and 10% sugar beet pulp and incubated at 40 °C for 72 h. Subsequent cultures were done in liquid and solid media until isolation of colonies. Screening for cellulase-producing bacteria was carried out on nutrient agar plates containing 1% carboxymethyl cellulose. Selected isolates were characterized based on morphological and physiological characteristics. A total of 26 bacterial strains were isolated, and 34 % were identified as cellulase producers. Diameters of clearing zones (cellulose hydrolysis) around colonies presented more than 20 mm. Moreover, 54 % of these isolates grew up to 25 % NaCl and 77 % fermented glucose. Additionally, 39 % of the strains grew up to 45 °C, 19 % of strains to pH 3, and 77 % of strains to pH 10.

Conclusions

Sugar beet pulp can be used as a selective substrate for the isolation of versatile bacteria producing highly active cellulases, which could have potential industrial applications

FEMS7-0583
Biotechnology / Synthetic Biology / Systems Biology

CONTINUOUS ABATEMENT OF METHANE COUPLED WITH ECTOINE PRODUCTION BY METHYLOMICROBIUM ALCALIPHILUM 20Z: A STEP FURTHER TOWARDS GHG BIOREFINERIES.

S. Cantera[1]
[1]*University of Valladolid, Chemical engineering and Environmental Technology, Valladolid, Spain*

Backgrounds

The implementation of environmentally friendly CH_4 abatement biotechnologies is not cost-efficient when applied to dilute off-gas emissions such as those from landfills or coal mines. In this sense, the production of high-added value products during CH_4 abatement can become a profitable approach to mitigate climate change.

Objectives

The research here presented investigated for the first time the valorization of dilute CH_4 emissions coupled with the production of ectoine, a microbial molecule with a high retail value in the cosmetic industry (approximately \$1300 kg^{-1}).

Methods

In this context, on a first attempt, the influence of Cu^{2+}, NaCl and CH_4 concentration, as well as temperature, on ectoine production by *Methylomicrobium alcaliphilum 20Z* was evaluated batchwise in gas-tight bioreactors. The results obtained showed that high concentrations of NaCl and CH_4 resulted in increased intra-cellular ectoine yields and high Cu^{2+} concentrations (50 µM) unexpectedly promoted the excretion of ectoine to the culture broth, despite this methanotroph has not been previously classified as an ectoine-excreting strain. The continuous abatement of CH_4 combined with the production of extra and intra-cellular ectoine was further evaluated in 6 stirred tank reactors.

Conclusions

The results obtained in these studies supported the technical viability of a new generation of GHG biorefineries and confirmed that high concentrations of NaCl resulted in higher steady state intra-cellular ectoine yields. In addition, an increase in Cu^{2+} concentration enhanced CH_4 abatement by a factor of 2 and promoted the excretion to the cultivation broth of 20 % of the total ectoine synthesized under continuous operation.

FEMS7-0402
Biotechnology / Synthetic Biology / Systems Biology

AZOARCUS SP. CIB AS A BIOREACTOR FOR PRODUCTION OF METALLIC NANOPARTICLES

M. Carmona[1], *H. Fernández-Llamosas*[1], *L. Castro*[2], *M.L. Blázquez*[2], *E. Díaz*[1]
[1]*Centro de Investigaciones Biológicas-CSIC, Environmental Biology, Madrid, Spain*
[2]*Facultad de Químicas- Universidad Complutense de Madrid,*
Material Science and Metallurgical Engineering Department, Madrid, Spain

Backgrounds

The β-Proteobacterium *Azoarcus* sp. CIB displays several features of great environmental interest. Thus, the strain CIB has the ability to degrade toxic aromatic compounds, e.g., toluene and *m*-xylene, and to live as an endophyte of rice (1). Recently, *Azoarcus* sp. CIB was shown to tolerate high concentrations of selenite and produce nanoparticles of selenium (2).

Objectives

Here we show that the metal resistance ability of strain CIB extends to other metalloids such as the oxyanion tellurite. The resistance mechanism appears to be the combination of at least three different processes that are interconnected along the cell growth, i.e., an active transport system, the methylation to volatile forms, and the reduction of the oxyanion to elemental metalloid.

Methods

Using different techniques such as TEM, SEM and EDX analyses, we have demonstrated that strain CIB transforms tellurite to less toxic nanorods of tellurium whose size ranges between 50-250 nm long and 5-10 nm width.

Conclusions

These results display the potential of the CIB strain as a suitable biocatalyst for bioremediation of toxic aromatic compounds and heavy metals and metalloids, and as a green biofactory for the production of metallic nanoparticles with biotechnological interest.

References:1. Fernández- Llamosas et al. (2014) PLoS ONE 23:e110771
2. Fernández-Llamosas et al. (2016) Microb. Cell Fact. 15:109

FEMS7-2379
Biotechnology / Synthetic Biology / Systems Biology

THE SOS-RESPONSE CONTROLS INTRINSIC CEPHALOSPSRIN RESISTANCE IN ENTERORCOCCI

L. Carrilero[1], S. Matrat[1], D. Thomas- Lopez[1], B. González-Zorn[1]
[1]Universidad Complutense de Madrid, Animal Health, MAdrid, Spain

Backgrounds

Enterococcus faecalis is a bacterium well known for causing multidrug resistant nosocomial infections. This pathogen exhibits intrinsic resistance to cephalosporins, which, despite all efforts, has not been fully understood. Clinically this is very significant, because cephalosporin treatment constitutes a risk factor for subsequent enterococcal infections.

Objectives

As the SOS response has been shown to be involved in both antimicrobial resistance and virulence of different bacteria we intend to investigate its implication on the resistance profile of *E*. *faecalis*, with special interest on cephalosporin resistance.

Methods

Several mutants in the SOS response in two different genetic backgrounds, JH2-2 and V583, were constructed. Further, a transposon mutant library in order to identify the genetic determinants involved in the phenomenon. MICs were determined using EUCAST methodology.

Conclusions

We discovered that *E. faecalis* has a functional SOS response and assessed its involvement in the sensitization of *E. faecalis* to methoxyiminocephalosporins. Within our transposon mutant library, we identified two glutamine transporters and the *cop* operon as components possibly implicated in decreasing the MIC of *E. faecalis* to cephalosporins. One of the glutamine transporters identified in our library is under the regulation of CroR/S, which has been previously described to play a role in cephalosporin resistance. Additionally, we found that the presence of copper further completely sensitizes *E. faecalis* to cephalosporins. Unraveling the mechanism of intrinsic resistance to cephalosporins in *E faecalis* paves the way for future novel treatment options against this important nosocomial pathogen.

FEMS7-1112
Biotechnology / Synthetic Biology / Systems Biology

FUEL FROM RUBBISH: ETHANOL PRODUCTION FROM ORGANIC MUNICIPAL SOLID WASTE USING A NOVEL ENRICHED MICROBIAL COMMUNITY

P. Carrillo-Barragan[1], *N.D. Gray*[1]
[1]*Newcastle University, Civil Engineering and Geosciences, Newcastle, United Kingdom*

Backgrounds

Lignocellulosic ethanol (EtOH) is a sustainable alternative to fossil transport fuels. Research has been focused on genetically engineering single microorganisms to fully convert lignocellulosic substrates into EtOH. This approach faces instability, narrow operational conditions and is susceptible to inhibition by contaminants such as those found in waste substrates.

Objectives

We propose the assembly of a robust microbial community from natural environments to transform Organic Municipal Solid Waste (OMSW) into EtOH with the manipulation of physical variables as a means to direct this process.

Methods

Environments where lignocellulose degradation occurs were sampled and used as inocula in microcosms with acid/steam pre-treated OMSW as substrate, cultured at a range of conditions (aerobic vs anaerobic, 4-7 pH values) at 20°C. The fermentation products were monitored using GC-FID and IC. Bacterial DNA was extracted at different time points and Ion torrent was used to sequence the 16Sr RNA amplicon libraries. Pipeline analyses were done using Mothur and Qiime software.

Conclusions

EtOH was the major product in rumen and sludge inoculated systems, both generating their highest EtOH concentrations (~35mM EtOH) when growing at different pH values (7 and 5, respectively) at both initial aerobic and anaerobic conditions. Microcosms inoculated with the combination of both inocula, generated EtOH as the major fermentation product under the range of pH 5 to 7. In the combined community, *Pseudomonas* sp. and *Clostridium sp.* were the most abundant species. Ongoing test are aimed to unravel the mechanisms behind the new community EtOH production to help understand and optimize this process by manipulating physical conditions.

FEMS7-0339
Biotechnology / Synthetic Biology / Systems Biology

COMPARATIVE ANALYSIS OF EXTRA VERSUS INTRACELLULAR HYDROLYSIS ON CELLOBIOSE FERMENTATION BY SACCHAROMYCES CEREVISIAE

M. Casa Villegas[1], *J. Marín-Navarro*[1], *J. Polaina*[1]
[1]*Instituto de Agroquímica y Tecnología de Alimentos, Biotecnología de alimentos, Paterna, Spain*

Backgrounds

Cellobiose is, besides glucose, a final product of cellulolytic enzyme complexes and therefore a key sugar for the production of second generation bioethanol and other fermentation products from cellulosic material. To overcome the inability of *Saccharomyces cerevisiae* to utilize cellobiose, two main approaches have been used: i) expression in the yeast of a secreted β-glucosidase that hydrolyzes cellobiose extracellularly; ii) cellobiose transport and concomitant intracellular hydrolysis, mediated by combined expression of a cellobiose permease and an intracellular β-glucosidase.

Objectives

Our aim is to compare the efficiency of the two approaches used for cellobiose fermentation by *S. cerevisiae* and predict conditions at which the use of one or the other method could be more practical.

Methods

We have cloned, expressed in *S. cerevisiae* and characterized the function of different heterologous genes encoding intracellular β-glucosidases and cellobiose permeases, to find out pairwise hydrolase/permease associations that enable efficient cellobiose fermentation by the host yeast strain. The performance of these strains was compared with a previously described *S. cerevisiae* strain that ferments cellobiose very efficiently (comparable to glucose) due to the expression of an extracellular β-glucosidase from *Saccharomycopsis fibuligera*.

Conclusions

The yeast strain expressing extracellular β-glucosidase, in medium with cellobiose as the carbon source, showed better growth and fermentation (ethanol production) than any strain that carried intracellular hydrolysis of the disaccharide. The only scenario that we envision where the use of the last approach would be advantageous is simultaneous saccharification and fermentation by cocultures of the fermenting yeast with a cellulolytic microorganism.

FEMS7-2987
Biotechnology / Synthetic Biology / Systems Biology

NUMERICAL OPTIMIZATION OF LIPASE PRODUCTION BY SOLID-STATE FERMENTATION WITH ASPERGILLUS FUMIGATUS

G.L. Castiglioni[1], R. Pérez Torrado[2], L. Alberto Oliveira Rocha[3], J. Alberto Vieira Costa[4]
[1]Universidade Federal de Goiás, Food Engineering, Goiania, Brazil
[2]Instituto de Agroquímica y Tecnología de Alimentos, Molecular biology, Valencia, Spain
[3]Universidade Federal do Rio Grande do Sul, Departamento de Engenharia Mecânica, Porto Alegre, Brazil
[4]Universidade Federal do Rio Grande, Escola de Química e Alimentos, Rio Grande, Brazil

Backgrounds

Capes

Objectives

The aim of this study was to model and numerically optimize lipase production through solid-state fermentation with filamentous fungus *Aspergillus fumigatus* using rice industry byproducts as substrate under different aeration conditions.

Methods

Strain of filamentous fungus *Aspergillus fumigatus* was used in experiments.
Cultivations were conducted in system described by Castiglioni et all [13]. The growth medium was composed of nutrient solution, rice hulls and defatted rice bran.
Soybean or diesel oils were added in separate experiments at a ratio of 1% (w/w), as an additional carbon source, for further comparison with experiments without their addition. Besides cultures without air supply, aerations of 40, 60, 120 and 200 $mL_{air}.g_{medium}^{-1}.h^{-1}$ were evaluated, aiming to study a wide range of this variable.
Preliminary tests were carried out using different mathematical functions, which were reparameterized for different equations. For optimum adjustments, determination coefficients were calculated. The coefficients of equations were numerically calculated by the method of least squares, and experimental and predicted results were statistically analyzed using univariate variance for significant differences.
Optimal lipase activity values, obtained by the model, were collected for each aeration condition studied. The results have been maximized in relation to aeration to determine the conditions that showed the best lipase production results. The model was validated by performing a new experiment, but under conditions different from those used to construct the model. The condition chosen for the model validation was 90 $mL_{air}.g_{medium}^{-1}.h^{-1}$.

Conclusions

The results of the model developed follow the trends of experimental results, showing similar lipolytic activities. The result of lipase production optimization was 107.56 $U.g^{-1}$ using 83 $mL_{air}.g_{medium}^{-1}.h^{-1}$ during 102 h of fermentation. The validation of mathematical model showed no significant differences from the predicted results, which ensures reliability as a new alternative to optimize the solid-state fermentation process with *Aspergillus fumigatus* using rice byproducts.

FEMS7-2815
Biotechnology / Synthetic Biology / Systems Biology

CRUDE LIPID EXTRACTS OBTAIN FOR IDENTIFICATION OF MYCOBACTERIA'S PHENOLIC GLYCOLIPID

B. Chavarro-Portillo[1,2], *Z. Rivera-Monroy*[2], *C.Y. Soto*[2], *M.I. Guerrero*[1]
[1]*Hospital Universitario Centro Dermatológico Federico Lleras Acosta E.S.E, Línea Hansen, Bogotá, Colombia*
[2]*Universidad Nacional de Colombia, Departamento de Química- Facultad de Ciencias, Bogotá, Colombia*

Backgrounds

M. leprae possesses the specific-antigenic known as phenolic glycolipid-1 (PGL-1) that mediates the interaction between *M. leprae* and Schwann cells. We previously developed a ***Bio-Nano-technological tool*** for PGL-1 detection, which will be used to detect antibodies against PGL from other skin disease-producing mycobacteria, such as *M. tuberculosis, M. marinum* and *M. ulcerans.* For this goal, PGL patterns from these mycobacteria should be identified and isolated.

Objectives

To design a methodological approach for the identification and purification of PGL from *M. tuberculosis, M. marinum* and *M. ulcerans,* by reverse-phase HPLC.

Methods

Mycobacteria were grown in Middlebrook 7H9 broth for 28 days at 37 °C, and crude-lipid extracts were obtained by extraction using the solvent system CH_3OH: H_2O. PGL was then separated by reverse phase HPLC, using a C18 column to bind the phenol group of the PGL structure, and two mobile phases (acetonitrile: water and methanol: water) were evaluated.

Conclusions

The mobile phase acetonitrile: water solvent systems, from 5:95 to 100: 0 in 40 min, allowed better peak resolution retention times (from 6.9 to 34.3 minutes) compared to those obtained using the mobile phase methanol: water. This is a highly sensitive and specific method that can be applied for PGL detection and purification from mycobacterial species of clinical interest, which produces enough amounts of PGL for detecting anti-PGL antibodies in clinical samples.

FEMS7-0971
Biotechnology / Synthetic Biology / Systems Biology

USING COMPUTATIONAL SYSTEMS BIOLOGY TO UNDERSTAND THE IMPACT OF GENOMIC DIVERSITY IN THE MYCOBACTERIUM TUBERCULOSIS COMPLEX

A. Chiner-Oms[1], F. Gonzalez-Candelas[1], I. Comas[2]
[1]FISABIO-Universitat de Valencia, Unidad Mixta Infección y Salud Pública, Valencia, Spain
[2]Biomedicine Institute of Valencia, Tuberculosis Genomics Unit, Valencia, Spain

Backgrounds

Species of the *Mycobacterium tuberculosis* complex (MTBC) kill more people every year than any other infectious disease. Because of its global distribution and parallel evolution with the human host, the bacteria is not genetically homogeneous. The observed genetic heterogeneity has relevance at different phenotypic levels, including epidemiological dynamics. However, current datasets have focused in the laboratory reference strain H37Rv.

Objectives

Our main objective is to define how the genetic variability identified in a global collection of isolates affect the biological networks derived from H37Rv experimental data.

Methods

First, we have constructed computational models to grab the expression dynamics of H37Rv genes. We have found that many of those transcription factors are deleted or likely dysfunctional across the MTBC. In accordance, we failed to predict expression changes in strains with a different genetic background when compared with experimental data.
Second, we have mapped about 200000 polymorphisms extracted from 9000 clinical strains on the interactome of the bacteria. We observed that the core of the interactome is more conserved than the periphery. A higher proportion of high impact mutations are in clusters of proteins located on the rim of the interactome than those in the center. The pattern of these mutations impacting the interactome is different for each lineage.

Conclusions

The biological networks of the pathogen are not fully conserved and there is variability among the different lineages. In order to obtain reliable models with which to make predictions there is a need of experimental data from representative samples comprising the natural diversity of the MTBC.

FEMS7-1528
Biotechnology / Synthetic Biology / Systems Biology

TUNING SYNTHETIC GENE EXPRESSION FOR ENHANCED PRODUCTION OF HETEROLOGOUS MEVALONATE IN ESCHERICHIA COLI

B.H. Choi[1], S.J. Kwon[1], H.J. Kang[1], P.C. Lee[1]
[1]Ajou University, Molecular Science and Technology, Suwon, Republic of Korea

Backgrounds

Isopentenyl diphosphate (IPP) is building block for isoprenoids, which are one of the most abundant natural secondary metabolite, including carotenoids and terpenoids. Most prokaryotes synthesize IPP by 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, while most plants, eukaryotes and some Gram-positive bacteria synthesize by mevalonate (MVA) pathway.

Objectives

Here we report cloning and reconstruction of three upstream mevalonate (MVA) pathway genes of novel bacterium *Enterococcus Kingsejongenesis*, which was isolated from a South Polar Brown Skua, in *Escherichia coli* for the production of MVA.

Methods

Each gene encoding the three upstream MVA pathway enzymes, HMG-CoA synthase (*mvaS*) and Thiolase/HMG-CoA reductase (*mvaE*), was modularized as an individual synthetic expression unit, and then was functionally expressed with a single or assembled module in *E.coli*. For balanced and fine-turned protein expression in metabolic pathway, expression levels of *mvaS* and *mvaE* were regulated by synthetic expression cassette (SEC) library. Randomizing promoter strength and ribosome binding site (RBS), simultaneously, was employed to diversify expression of mvaS and mvaE protein specifically in *E.coli*.

Conclusions

In this study, functional expression module of MVA pathway was constructed by modularization of each gene. The MVA pathway module from *E.kingsejongenesis* produced 1.6-fold higher mevalonate compared with *Enterococcus faecalis* in *E.coli*. Furthermore, fed-batch fermentation of the recombinant *E.coli* under the newly designed promoter and RBS module resulted in the 1.5-fold enhanced MVA production than that obtained by MVA pathway module from *E.kingsejongenesis* using *lac* promoter. The *E.coli* strain engineered in this study can serve as the basis for creating an alternative way for production of MVA.

FEMS7-2766
Biotechnology / Synthetic Biology / Systems Biology

CHEMICALLY INDUCED BACTERIAL GHOSTS AS AN SAFE AND EFFICIENT NON-VIRAL DNA DELIVERY VEHICLE IN VITRO

C.W. Choi[1], H.B. Noh[1], N. Vinod[1], S. Oh[1], S. Ji[1], S. Kim[1], H.J. Park[1]
[1]Paichai University, Department of Biology & Medicinal Science, Daejeon, Republic of Korea

Backgrounds

Gene therapy represents promising method to treat diseases by delivering a therapeutic gene within the target cell. To deliver DNA into target cell, a carrier is required. Bacterial ghosts (BGs) are novel and non-living empty bacterial cell envelopes mainly derived from Gram-negative bacteria. The plasmid DNA immobilized in BG system has been shown to be a safe and promising technology for gene delivery and indicating alternative to current viral and bacterial gene delivery system.

Objectives

In this study, we used chemically induced BGs from *Salmonella typhimurium* for the first time as a delivery carrier for green fluorescent protein (GFP) gene to murine macrophages.

Methods

The S. typhimurium ghosts (STGs) were produced using the MIC of NaOH and loaded with minicircle DNA containing GFP gene by diffusion method. Real time PCR analysis confirmed that BGs loaded with minicircle plasmid carrying the GFP gene. *In vitro* studies after transfection showed that the STGs more efficiently delivered the minicircle plasmid within the macrophages. The expression of GFP gene was determined by RT-PCR and qPCR. Moreover, we examined uptake of the STGs loaded with minicircle DNA by confocal laser scanning microscopy.

Conclusions

We have shown that BGs loaded with minicircle DNA efficiently delivered and taken up by macrophages. When compared to minicircle DNA alone, STGs loaded with minicircle DNA was increased transgene expression level. These studies demonstrate for the first time potential of chemically-induced BGs could be used as a novel delivery vehicle and targeting carriers for gene therapy.

FEMS7-2777
Biotechnology / Synthetic Biology / Systems Biology

PROTECTIVE IMMUNITY AGAINST LISTERIOSIS IN RATS, PROVIDED BY CHEMICALLY-INDUCED LISTERIA MONOCYTOGENES BACTERIAL GHOSTS (LMGS)

C.W. Choi[1], S. Ji[1], S. Oh[1], H.B. Noh[1], K. Seongdae[1], H.J. Park[1], N. Vinod[1]
[1]Paichai University, Department of Biology & Medicinal Science, Daejeon, Republic of Korea

Backgrounds

Listeriosis, caused by the gram-positive, facultative intracellular bacterium, is one of the leading causes of food borne infections in the industrialized world and an etiologic agent of listeriosis to humans with a high mortality rate. Most common clinical feature includes meningitis or septicemia in children and the elderly. Therefore, The development of Listeria vector candidates by attenuation of its virulence is needed for safety considerations.

Objectives

To investigate the LMGs as a potential vaccine candidate, mice were divided into three groups; non-vaccinated control group and subcutaneously immunized groups with HCl-induced LMGs and NaOH-induced LMGs, Most importantly, bacterial loads in both of LMGs-immunized groups were significantly lower than non-immunized control group after virulent *Lm* challenge. These results suggest that immunization with LMGs induces both humoral and cell-mediated immune responses and provides protection against virulent *Lm*.

Methods

Listeria monocytogenes bacterial ghosts (LMGs) were generated by chemically induced lysis and the method is based on minimum inhibitiory concentration of HCl and NaOH. Among LMGs samples, To investigate the LMGs as a potential vaccine candidate, mice were divided into three groups; non-vaccinated control group and subcutaneously immunized groups with HCl-induced LMGs and NaOH-induced LMGs, respectively.

Conclusions

Non-living LMGs has been successfully generated by MICs and the approach is rapid and cost-effective when compared to other known methods. Interestingly, the present strategy may open the door produce BGs from Gram-positive bacteria. Most importantly, we have shown that immunization with LMGs induced significant humoral and cellular immune responses and provided strong protection against virulent challenge in BALB/c mice.

FEMS7-3029
Biotechnology / Synthetic Biology / Systems Biology

LEPTOSPIRILLUM FERRIPHILUM; GENOMIC, TRANSCRIPTOMIC, AND PROTEOMIC CHARACTERIZATION OF AN ACIDOPHILE MODEL SPECIES

S. Christel[1], M. Herold[2], S. Bellenberg[3], M. El Hajjami[4], A. Buetti-Dinh[5], I. Pivkin[5], W. Sand[6], P. Wilmes[2], A. Poetsch[7], M. Dopson[1]
[1]Linnaeus University, Ecology and Evolution in Microbial Model Systems, Kalmar, Sweden
[2]University of Luxembourg, Luxembourg Centre for Systems Biomedicine, Luxembourg, Luxembourg
[3]University Duisburg-Essen, Aquatic Biotechnology, Essen, Germany
[4]Ruhr University Bochum, Plant Biochemistry, Bochum, Germany
[5]Università della Svizzera Italiana, Institute of Computational Science, Lugano, Switzerland
[6]Donghua University, College of Environmental Science and Engineering, Shanghai, China
[7]Plymouth University, School of Biomedical and Healthcare Sciences, Plymouth, United Kingdom

Backgrounds

In recent decades, bioleaching of sulfidic ores has been firmly established as an environmentally sustainable alternative to conventional metal extraction methods such as roasting and smelting. By using this technique, environmental pollution is kept at a minimum, as only acidophilic organisms catalyze the dissolution of the sulfide mineral, releasing the incorporated metals. Among the microorganisms in bioleaching heaps, the autotrophic iron oxidizer *Leptospirillum ferriphilum* is one of the most abundant.

Objectives

Despite its importance in biomining and other metal rich and low pH environments, no complete genome sequence of this model species' type strain has been available. However, for further study of the organism and its role in bioleaching communities, a full genome is essential as a reference for proteomic and transcriptomic investigation.

Methods

In this study, Pacific Bioscience (PacBio) single molecule real time sequencing was utilized to provide a closed, circular *L. ferriphilum*^{T} DSM14647 genome of high quality for future use as a publicly available reference. Concurrently, continuous cultivation of axenic *L. ferriphilum*^{T}, grown on its sole substrate ferrous iron, was performed to establish a proteome baseline, and sequence a transcriptome baseline via Illumina HiSeq sequencing technology. Likewise, cells were incubated in batch cultures containing chalcopyrite ($CuFeS_2$; the world's most abundant copper mineral) as a substrate, to investigate *L. ferriphilum*'s resistance to low pH, elevated levels of copper, and oxidative stress caused by the mineral and released heavy metals.

Conclusions

The data made available by this project will serve as a point of reference in future studies on *L. ferriphilum*^{T} and its role in environment and biotechnology.

FEMS7-0291
Biotechnology / Synthetic Biology / Systems Biology

ELECTROSPUN OF SILK FIBROIN IMMERSED ANOECTOCHILUS ROXBURGHII EXTRACTIONS (CUST903) FROM PLANT FACTORY SYSTEM FOR ANTIOXIDANT AND ANTIBACTERIAL WOUND DRESSING

J.T. Kuo[1], Y.C. Chang[1], Y.C. Chung[1]
[1]China University of Science and Technology, Department of Biological Science and Technology, Taipei city, Taiwan R.O.C.

Backgrounds

CUST903 was extracted by ethanol from *Anoectochilus roxburghii* from Plant Factory production system. In the study, silk fibroin (SF) mat was prepared by electrospinning technique, followed by immersing in solution of CUST903 to evaluate the feasibility of these mats for wound dressing. SF nanofibers provided a better level of equilibrium water content (EWC) and water vapor transmission rate (WVTR).

Objectives

Antioxidant activity of CUST03-loaded SF mats was measured by the DPPH assay and increasing with its concentrations. They exhibited strong antioxidant DPPH radical scavenging activity with IC50 value of 9.8 and 23.6 μg/ml for ascorbic acid and alcoholic leaves extract respectively. The absorbance for reducing power was found to be 0.0410, 0.0932 for ascorbic acid and alcoholic leaves extract respectively. In additions, Cytotoxicity of CUST03-loaded SF mat was evaluated using L929 and A375-S2 fibroblasts through the MTT assay. The antibacterial activity of CUST903-loaded SF mat was evaluated against *E coli* and *S. aureus only 0.13ug/ml*.

Methods

Silkworm cocoons were boiled in an aqueous solution of 0.02M Na2CO3 for 30 min to remove the sericin and then rinsed thoroughly with distilled water. The degummed silk fibroin was then dissolved in a mixture of CaCl2 : C2H5OH : H2O (molar ratio = 1:2:8) at 70°C for 6 h and dialyzed against distilled water for 3 days with successive water changes to remove salt. The Anoectochilus roxburghii was made by plant Factory system which was controlled by light source, temperature and humidity conditions.

Conclusions

Through characterization of physical properties, stability testing, and biocompatibility, the CUST903-loaded SF mat exhibited potential for Antioxidant and antibacterial wound dressing applications.

FEMS7-1368
Biotechnology / Synthetic Biology / Systems Biology

POLYMORPHISM IN THE REGULATORY REGION OF THE CGYPS1 AND CYYPS7 GENES ENCODING FOR YAPSINS OF CANDIDA GLABRATA

E. Cortés-Acosta[1], J.A. Ibarra-García[1], H.C. Ramírez-Saad[2], M.L. Villa-Tanaca[1], C.H. Hernández-Rodríguez[1]
[1]Instituto Politécnico Nacional-ENCB, Microbiología, Mexico City, Mexico
[2]Universidad Autónoma Metropolitana-UAM-X, Sistemas Biológicos, Mexico City, Mexico

Backgrounds

Candida glabrata is an opportunistic fungus infecting mainly immunocompromised people. Their adherence capacity and exoenzymes contribute to damage to host cell. Particularly, the yapsins are a family of aspartyl proteases involved in maturation of proteins, cell wall function, and etcetera. Yapsin 1 and 7, encoded by *CgYPS1* and *CgYPS7* genes respectively, are a potential virulence factors.

Objectives

In this study, the diversity of regulatory regions and expression profiles of the both genes from *C. glabrata* clinical strains were compared.

Methods

The polymorphism in regulatory regions of both yapsin genes were recognized by DGGE (Denaturing Gradient Gel Electrophoresis) migration profiles. The sequence analysis of regulatory regions revealed that the distribution of Transcription Factor Binding Sites (TFBS) were similar, although some TFBS were not universally distributed. The quantitative expression of *CgYPS7* genes of different *C. glabrata* strains in rich and poor media was estimated by RT-qPCR.

Conclusions

The CGL8 strain, which contains TFBS for a carbon source-responsive element, had a high expression of *CgYPS7* gene in YPD medium. However, the expression levels in YNB medium without nitrogen source of most the strains were, a result coherent with the presence of F$YMAT, a TFBS associated to nitrogen starvation conditions. Also, the CGL33 strain exhibited high expression levels of the *CgYPS7* gene in YNB without carbon source. The primary sequences of *CgYPS7* genes of *C. glabrata* strains are highly conserved among different strains, however the regulatory regions are polymorphic, harbor different TFBS arrays, and showed differential expression profiles.

FEMS7-2391
Biotechnology / Synthetic Biology / Systems Biology

"IN VITRO" ANTIMICROBIAL ACTIVITY OF THE HYDROALCOHOLIC EXTRACT OF LAFOENSIA PACARI

A.K.M. Santos[1], M.B.P.C. Camara[1], F. Costa Rosa[2], C. Nadine Cunha[3], M. Francyelle Costa[2], C.F. Antonio José[1], M. Georgiana Eurides de Carvalho[1], C. Fernando José Costa[1]
[1]IFMA, Química, São Luís, Brazil
[2]Faculdade Estácio de São Luís, Enfermagem, São Luís, Brazil
[3]LABCENTER, Controle de qualidade, Palmas, Brazil

Backgrounds

Crude extracts or isolated plant coumpounds used in popular medicine can be the source of research for new antimicrobials. Among these plants, there is *Lafoensia pacari*, popularly known as "mangaba brava", easily found in the brazilian "Cerrado" (tropical savannah ecoregion) area. Traditionally, it is used by popular medicine in the form of infusions and leaf and bark maceration for treating diseases

Objectives

To verify the "in vitro" antimicrobial activity of the hydroalcooholic extract of *L. pacari.*

Methods

The vegetal species *Lafoensia pacari* was collected in the village of Contrato, in the city of Morros-MA, under the localization S 02° 54' 47,6" e W 043° 55' 30,1". The barks were dried and posteriorly the extract was obtained using the extracting solvent hydroalcoholic 70% in the proportion of 1:3 (m/v). Afterwards, the extract was submitted to the rotative evaporator under reduced pression, with the temperature controlled up to 50°C, obtaining the final concentraion of 2,9mg/mL. In order to verify the antimicrobial activity, the techniques of agar diffusion and microdilution with the following standard strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 14053)*, Candida krusei* (ATCC 6258) *e Candida parapsilosis* (ATCC 22019).

Conclusions

The extract of *L. pacari* presented only the bacteriostatic effect over the evaluated strains. However, concerning its antifungal activity, we observed the fungicide capacity on all the *Candida* gender strains, presenting the following MFC: 0,36 mg/mL, 0,18 mg/mL e 0,09 mg/mL to *C. krusei, C. albicans* e *C. parapsilosis*,

FEMS7-2536
Biotechnology / Synthetic Biology / Systems Biology

ANTIMICROBIAL POTENTIAL OF ETHYL ACETATE FRACTION OF LUEHEA CANDICANS AGAINST BACTERIA AND FUNGI

M. Bispo Pinheiro Camara[1], A.J. Cantanhede Filho[1], F.J. Costa Carneiro[1], T. Daniele Val Lima[2], F. Costa Rosa[3], F. Costa Moraes[3], G. Eurides de Carvalho Marques[1]
[1]IFMA, Química, São Luís, Brazil
[2]Faculdade Estácio de São Luís, Química, São Luís, Brazil
[3]Faculdade Estácio de São Luís, Enfermagem, São Luís, Brazil

Backgrounds

Popular medicine empirically diffuses the use of plants's leaves of the genus *Luehea* to treat various diseases. Among the species, stands out the *Luehea candicans*, a brazilian native plant with few reports in the literature about your antimicrobial potential.

Objectives

To inquire the antimicrobial potential of the ethyl acetate fraction of *Luehea candicans* against bacteria and fungi.

Methods

Luehea candicans (Titulaceae) was collected in the Contrato's Village in the city of Morros/MA, under the location S 020 55 '21.0' 'and W 0430 55' 34.7 ''. The botanical identification of the species was registered under the number 4586 that is deposited in the Herbarium of the State University of Maranhão. The weeds were dried and then the crude extract was extracted using 70% hydroalcoholic solvent in the ratio of 1: 3 (m / v). From the ethyl acetate phase, it was parted with dichloromethane again, gaining a ethyl acetate fraction at a concentration of 9.77 mg/mL. The antimicrobial potential was verified in the agar drilling and microdilution techniques using the following species: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 14053), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019) and clinical samples of *C. glabrata* and *C. albicans.*

Conclusions

Only in the microdilution technique was it possible to verify the intense antimicrobial potential of fraction evaluated, highlighting just 1.22 mg/mL CFM and 2.44 mg / mL for clinical samples of *C. glabrata* and *C. albicans* respectively. In the bacteria only inhibitory effect was observed.

FEMS7-2955
Biotechnology / Synthetic Biology / Systems Biology

COMBINATION OF FOOD WASTES FOR AN EFFICIENT PRODUCTION OF NISIN IN REALKALIZED FED-BATCH CULTURES

M. Costas Malvido[1], *E. Alonso González*[1], *N. Pérez Guerra*[1]
[1]*Universidad de Vigo, Department of Analytical and Food Chemistry, Vigo, Spain*

Backgrounds

Nisin is a bacteriocin produced by *Lactococcus lactis* strains, with a broad spectrum of antibacterial activity against undesirable spoilage bacteria and food-borne pathogens including *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum*. This bacteriocin is innocuous, stable to heat at low pH levels and sensitive to the gastric proteinases. For these characteristics, nisin is used as a safe preservative for different foods in many countries. However, considerably higher productions of nisin have been obtained in fed-batch cultures in comparison to the batch mode.

Objectives

To obtain increased nisin production by *L. lactis* CECT 539 at low production cost

Methods

Realkalized fed-batch cultures in diluted whey (DW). The first and third cultures were fed with mixtures of whey (W, 51.35 g of lactose/L) and a 400 g/L concentrated glucose (CG), or with a concentrated mussel processing waste (CMPW, 101.72 g of glucose/L) and CG, respectively. The second and fourth cultures were respectively performed under the same conditions as in the first and third fermentations. However, these cultures were supplemented with W plus a 2 % (w/v) yeast extract (WYE2) and CG (second culture), or with CMPW plus a 2 % (w/v) yeast extract (CMPWYE2) (fourth culture) after sample extractions at 132 and 168 h, respectively. From these times, each culture was fed with mixtures of WYE2 and CG, or CMPWYE2 and CG, respectively

Conclusions

The third (223.98 BU/mL) and fourth (350.61 BU/mL) cultures provided higher nisin concentrations than the first (108.00 BU/mL) and second (158.53 BU/mL) fermentations

FEMS7-2223
Biotechnology / Synthetic Biology / Systems Biology

ACTIVATION OF SILENT GENE CLUSTERS IN FUNGI AS A TOOL FOR ANTIBIOTIC DRUG DISCOVERY

L. Cubilla Rios[1], *M.M. Aguilar-Pérez*[2]
[1]*Universidad de Panama, Química Orgánica, Panama, Panama*
[2]*Universidad de Panama, Laboratorio de Bioorgánica Tropical, Panama, Panama*

Backgrounds

The indiscriminate use of the current antimicrobials, the microbial capability to develop resistance and the existence of fewer effective antibiotics against certain infections make the discovery of new antibiotics an urgent priority. Endophytic fungi are capable of producing secondary metabolites with antibacterial activity as well as reacting to changes in culture conditions that could activate silent gene clusters. Thus, the ability to enhance the chemical diversity produced by the fungi will increase the possibility of uncovering new antibacterial activities.

Objectives

This research pursues the activation of silent gene clusters to increase the antibacterial spectrum of two fungal strains, *Pestalotiopsis mangiferae* and *P. microspora.*

Methods

In the first phase, strains were grown in five different **media** (e.g. PDA, MEA); second step a **chemical agent** (e.g. Arginine, $CuSO_4$) prior to inoculation onto the selected media. In the third phase, changes in **pH** were used as elicitors, with buffers being used to stabilize the pH (4.0, 4.6, and 5.6) of the media. Finally, changes in the incubation **temperatures** (e.g. 24 and 30 °C) were used as an elictor. An organic extract was obtained from each culture. The antibacterial activity was determined through a susceptibility test against 20 species.

Conclusions

Pestalotiopsis mangiferae produced the greatest inhibition spectrum, 11 bacteria, when MEA was used as a nutrient, $CuSO_4$ as chemical agent, the pH was 4.0, and the temperature 24 °C. In total 18 bacteria were inhibited by all the extracts obtained from the culture of this species. By *Pestalotiopsis microspora* in total 12 bacteria were inhibited. The antibacterial spectrum, in each case, has been clearly improved by using specific culture conditions.

FEMS7-0280
Biotechnology / Synthetic Biology / Systems Biology

STUDIES ON ANTI-NEOPLASTIC ENZYME - L-ASPARAGINASE ISOLATED FROM BACILLUS SPECIES

S. Dahikar[1]
[1]*Sanjivani Arts - Commerce and Science College - Kopargaon, Department of Microbiology, Kopargaon, India*

Backgrounds

L- asparaginase is enzyme acting on L- asparagine and is widely used as anticancer agent. The reason it is preferred for the purpose is it is biodegradable, non-toxic and can be administered at the local site quite easily.

Objectives

At present various microorganisms are used for production of L-asparginase. The main objectives of this study were to determine anti-neoplastic activity of enzyme - L-asparaginase from Bacillus species from soil.

Methods

In the present study, a total of five soil samples were collected from various places near to Kopargaon, from these soil samples 12 isolates were identified as *Bacillus licheniformis* (5)*, Bacillus alvei* (2), *Bacillus megaterium* (1), *Bacillus cereus* (1), *Bacillus circulans* (1) and *Bacillus subtilis* (2). The optimization study for the process parameters was given useful information about the production of the enzyme maximally. Potential L-asparginase producer isolates were showed the maximum activity at pH 7 and temperature at 37°C. After that the isolates were exposed to the UV treatment for 5, 10 and 15 min for strain improvement and among them Asp11 *(Bacillus subtilis)* was found useful strain for the commercial production of the L-Asparaginase. The purified enzyme of Asp11 was used for protein profile.

Conclusions

This highly potential species can be used for the commercial production of the enzyme which is having immense medical applications as it is one of the promising treatments for the threatening disease called as cancer.

FEMS7-1251
Biotechnology / Synthetic Biology / Systems Biology

ADJUSTMENTS IN THE LIPID PROFILE OF RHODOCOCCUS UNDER STRESSFUL CONDITIONS MAY BE USED TO IMPROVE BIOTECHNOLOGICAL PROCESSES

C.C. C. R. de Carvalho[1]
[1]*Instituto Superior Técnico, Department of Bioengineering- iBB-Institute for Bioengineering and Biosciences, Lisbon, Portugal*

Backgrounds

When bacterial cells are exposed to stressful conditions, such as the presence of organic compounds, antibiotics, salt or the lack of nutrients, they use a series of adaptive mechanisms to survive. Among these are modifications in the fatty acid (FA) composition of the cellular envelope.

Objectives

The studies aimed at improving biotechnological processes by application of bacterial adaptive mechanisms at the lipid level.

Methods

To determine the cell composition in FAs, after exposure to stress, total lipids were extracted, methylated and the resulting FA methyl esters were analyzed by gas chromatography (GC). Cell membrane composition was determined after chromatographically separating total lipids into neutral lipids, glycolipids and phospholipids. Biocatalysis and bioremediation of model compounds using adapted cells was followed by GC.

Conclusions

R.erythropolis cells changed their FA composition during adaptation to values of temperature, pH, salt and copper concentrations far from the optimum values for cell growth [1]. Following exposure to salt, high percentage of polyunsaturated FA were unexpectedly produced [2]. Alterations in the cell wall and membrane compositions and properties allowed these cells to tolerate compounds usually toxic to other bacterial strains such as terpenes used in biocatalysis, and hydrocarbons and aromatic compounds used in bioremediation [1,3]. The specific adaptive mechanisms also helped the cells to respond to fast environmental changes, and the establishment of the most tolerant cells in populations used in biotechnological processes.

[1] Res Microbiol (2012) 163:125-136 [2] Appl Microbiol Biotechnol (2014) 98:5599-5606 [3] Appl Microbiol Biotechnol (2009) 82:311-20

FEMS7-1866
Biotechnology / Synthetic Biology / Systems Biology

IMPACT OF DIETARY FAT CONTENT ON HOST SUSCEPTIBILITY TO LISTERIA MONOCYTOGENES INFECTION

V. de Las Heras[1], K. Govindarajan[1], S. Joyce[2], P. Casey[3], R. Rubio[4], P. Cotter[4], C. Hill[1], C. Gahan[1]
[1]University College Cork, School of Microbiology- APC Microbiome Institute, Cork, Ireland
[2]University College Cork, School of Biochemistry and Cell Biology- APC Microbiome Institute, Cork, Ireland
[3]University College Cork, APC Microbiome institute, Cork, Ireland
[4]APC Microbiome Institute, Moorepark Food Research Center- Teagasc, Fermoy- Cork, Ireland

Backgrounds

Currently the world is facing an escalation in cases of obesity, influenced by an increase in consumption of elevated levels of saturated fats and sugar, the so-called Western-diet. However the impact of these dietary changes on host susceptibility to foodborne pathogens such as *Listeria monocytogenes* is currently unclear.

Objectives

In this project, using an *in vivo* systems biology approach, we explore the host response to oral *Listeria monocytogenes* infection following murine exposure to defined rodent diets that mimic low-fat or high-fat (Western) diets.

Methods

Mice were fed a control chow diet, a low-fat diet (10% of caloric intake from fat) or a high-fat diet (45% of caloric intake from fat) throughout the experiment. The total bacterial load from the organs at 3 days post-infection was used to characterize *L. monocytogenes* infection. Microbiota analysis (16S DNA sequencing) and host transcriptome analysis were performed to explore the impact of the dietary fat content on the host.

Conclusions

Total bacterial loads from the organs harvested 3 days post-infection show an increased susceptibility to *L. monocytogenes* in mice receiving a high fat diet. This increased susceptibility to infection was associated with significant changes to the composition of the gut microbiota, physiological changes in the gut barrier and immune suppression caused by increased dietary fat content. The findings indicate that diet mediates physiological changes to the host, that can significantly influence susceptibility to infection with *L. monocytogenes*, suggesting that diet should be considered as an important factor in future models of *L. monocytogenes* pathogenesis.

FEMS7-1028
Biotechnology / Synthetic Biology / Systems Biology

UTILIZATION OF SACCHAROMYCES CEREVISIAE FOR BIOETHANOL PRODUCTION FROM TURNIP JUICE DISCARDS

E. Demiray[1], S. Ertuğrul Karatay[1], G. Dönmez[1]
[1]Ankara University, Biology, Ankara, Turkey

Backgrounds

Widespread usage of fossil fuels cause rapid depletion and there is several environmental consequences which triggers the researches for finding alternative sources. Among these sources bioethanol is one of the most important one because of its renewable and environmentally friendly properties. Because feedstock costs are the major part of bioethanol production, considerable work has been performed toward production of bioethanol using various kinds of feedstocks such as starch or lignocellulose.

Objectives

Turnip juice is made from black carrot and turnip. After turnip juice production; black carrots and turnips are not used and are disposed as a waste. In this study, we examined bioethanol production of turnip juice discards by *S. cerevisiae*. Some important parameters such as initial biomass loading, fermentation time and pretratment conditions were optimized.

Methods

Turnip juice discards were obtained from Kilikya Şalgam Co. and dried in the oven at the 70 ^{0}C. To obtain fermentable sugars; discards hydrolysed in the 1% H_2SO_4 (v/v). Yeast growth, initial and consumed sugar concentrations were monitored periodically. Bioethanol concentration was determinated by using gas chromotography.

Conclusions

Among the tested pretreatment methods the most effective one was acid hydrolysis with autoclaving. It was found in the study that the yeast consumed 2.31±0.06 g/L sugar when the ethanol concentration was 0.4 ±0.06 g/L at the end of 24 hours incubation time at pH 5 in the presence of 50 g/L biomass loading. In the present study we showed that bioethanol production from biomass of turnip juice discards can be increased by appropriate pretreatments.

FEMS7-1034
Biotechnology / Synthetic Biology / Systems Biology

USAGE OF CARROT POMACE FOR BIOETHANOL PRODUCTION BY KLUYVEROMYCES SP.

E. Demiray[1], *S. Ertuğrul Karatay*[1], *G. Dönmez*[1]
[1]*Ankara University, Biology, Ankara, Turkey*

Backgrounds

In today's world the widespread use of fossil fuels cause rapid depletion and several environmental consequences which triggers the researches for finding alternative fuels. Bioethanol is one the most important renewable source. Bioethanol as a renewable and clean energy source is getting more attractive. The ethanol also reduces carbon dioxide emissions of the vehicles up to 90% when it blended with gasoline. Hence investigation of more effective bioethanol production ways are important.

Objectives

Several different biotechnological applications have been investigated with *Kluyveromyces* sp. For this purpose in our current study we isolated novel osmotolerant yeast strains from biscuit factory wastes and they were used for bioethanol production. Among them *Kluyveromyces* sp. coded as isolate 8 had higher ethanol production capacity than the other isolates in the media containing carrot pomace as carbon source.

Methods

Carrot pomaces were supplied from BELSO Co. Ankara/Turkey. Pomaces were hydrolysed in 1% H_2SO_4 (v/v). Microbial growth, sugar consumption and bioethanol amounts were monitored periodically.

Conclusions

The highest bioethanol production was observed at pH 5 at the end of 12 hours incubation time. Furthermore there were significant amounts of ethanol production was seen at 24 hours incubation time for all tested pH values (4, 5, 6, 7). 8.13 g/L bioethanol was achieved at the end of 24 hours at pH 5.

In this study we showed carrot pomaces are suitable feedstocks for bioethanol production. Fermentation capacity of the *Kluyveromyces sp.* can be increased with different ways such as metabolic or evolutionary engineering.

FEMS7-1554
Biotechnology / Synthetic Biology / Systems Biology

CARBON MONOXIDE AS A COMPETITIVE INHIBITOR INDUCING REVERSIBLE INACTIVATION OF FE-FE HYDROGENASES BY NON-DISRUPTIVE BINDING AT ACTIVE SITE

K. Dixit[1], S. Singh[2], V. Kannaujiya[3], A. Rahman[1], A.K. Srivastava[1], S. Singh[1], R. Ettrich[4], S. Sundaram[1]
[1]University of Allahabad, Centre of Biotechnology, Allahabad, India
[2]BITS Hyderabad, Biological Sciences, Hyderabad, India
[3]Banaras Hindu University, Botany, Varanasi, India
[4]Institute of Microbiology Academy of Sciences, Center for Nanobiology and Structural Biology, Czechia, Czech Republic

Backgrounds

Hydrogenases efficiently catalyze the production and cleavage of molecular hydrogen. Their use in biotechnological applications is anticipated to make a remarkable contribution to future renewable fuel production. There are three types of hydrogenases: Fe-Fe, Ni-Fe and Fe-only hydrogenases.

Objectives

In this study, the Fe-Fe hydrogenases are majorly focused. The Fe-Fe hydrogenases of green algae (Chlorophytes) are also called “Chlorophyta type” hydrogenases and do not contain F-cluster. The presence of H-cluster at the active site is the main attribute of chlorophyte’s hydrogenase. Carbon monoxide (CO) is an effective inhibitor of Fe-Fe hydrogenases and so is oxygen (O_2).

Methods

The enzyme kinetics was studied for oxygen, carbon monoxide and CrHydA1 and K_m was calculated by Hyper32 software. K_m value 50μM, 22μM and 28 μM were recorded respectively for the inhibitors and the substrate suggesting CO as a potent non-destructive inhibitor, which can protect against oxygen damage.

Conclusions

Oxygen irreversibly destroys the enzyme active site causing potential loss of function. CrHydA1 is a substrate for hydrogenase enzyme and is important functionally for efficient enzyme activity. CO, being a competitive inhibitor of CrHydA1, binds to the iron atom of the 2Fe-H domain and is ordinarily, non-destructive, securing the Fe-S clusters to remain intact in the enzyme. CO has nearly two folds higher affinity for the active site of hydrogenase as compared to oxygen protecting the enzyme against damage from O_2.Here, we present a study of the mechanism by which O_2 irreversibly attacks the H-cluster, by in-silico techniques with the reversible inhibitor CO as a complementary binding substrate.

FEMS7-1230
Biotechnology / Synthetic Biology / Systems Biology

CHARACTERIZATION OF RESISTANCE GENES AGAINST VANCOMYCIN AND AMPICILLIN IN THE GUT MICROBIOTA OF BLATTELLA GERMANICA

R. Domínguez-Santos[1], I. Talón[1], C. García-Ferris[1], A. Artacho[2], A. Latorre[1]
[1]Institute for Integrative Systems Biology I2SysBio, University of Valencia, Paterna, Spain
[2]FISABIO Foundation, Centre for Advanced Research in Public Health, Valencia, Spain

Backgrounds

The research for new antibiotics against bacterial pathogens is an urgent priority of modern medicine, which has found that the number of bacteria resistant to multiple antibiotics is growing steadily, and therefore antibiotic resistance is recognized as one of the major challenges in public health. *Blattella germanica* is an omnivorous insect that harbour a rich and complex gut microbiota, similar in some characteristics to human gut microbiota, and may contain bacteria with many antibiotic-resistance genes that can be a reservoir to be transferred to humans.

Objectives

To study the composition of the gut microbiota and characterization of resistance genes against vancomycin and ampicillin.

Methods

We have worked with three synchronized adult populations of *B. germanica*. Two of them were treated with antibiotic (vancomycin or ampicillin, respectively) supplied *ad libitum* with water at 0.02% (w/v). The third population was used as a control.

Conclusions

The microbiota in the hindgut of *B. germanica* consists of a very large community of bacteria, and it varies considerably depending on the antibiotic supplied. In general, vancomycin increased the abundance of gram-negative bacteria, as in the phyla Proteobacteria, Fusobacteria and Planctomycetes and reduced the presence of gram-positive bacteria, as in the phylum Firmicutes. Furthermore, ampicillin increased representatives of Bacteroidetes and reduced the phylum Firmicutes. Vancomycin and ampicillin resistance genes encoding multidrug efflux pumps were detected mainly in the phylum Proteobacteria, while specific ampicillin resistance genes were identified in Firmicutes (Bacillales and Staphylococaceae) and Bacteroidetes. These results show that the microbiota of *B. germanica* responds to the effects of antibiotics.

FEMS7-3024
Biotechnology / Synthetic Biology / Systems Biology

LEVAN AND LEVANSUCRASES: THE ENZYME AND THE PRODUCING MICROORGANISMS, AN UPDATE.

A. Dominguez[1], Á. González-Garcinuño[2], A. Tabernero[3], M.A. Galan[2], E. Martin del Valle[2]
[1]Universidad de Salamanca, Microbiology and Geneticas, Salamanca, Spain
[2]Universidad de Salamanca, Department of Chemical Engineering, Salamanca, Spain
[3]Universidad de Salamanca, 1Department of Chemical Engineering, Salamanca, Spain

Backgrounds

Levansucrases have been studied only in a few microorganisms. The availability of cheaper and new methods of sequencing have allowed the detection new levansucrases in many bacteria

Objectives

Nowadays, levan synthesis and its biocatalysis is claiming attention due to its promising uses in biomedical field. The objective of our work is the analysis of the bacteria producing levansucrases.

Methods

Overall protein sequences obtained by BlastP comparisons against the database of non-redundant protein sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with the well characterized levansucrases of B. subtilis and Z. mobilis and based on high levels of similarity and/or a large functional homogeneity of the hits, we have detected the 685 strains of bacteria, The alignment of proteins was generated by using the Clustal Omega Program (http://www.ebi.ac.uk/services)

Conclusions

In this work we have analyzed the microbial levansucrases of sequenced bacteria concerning sequence homology, signal peptide, metal binding, etc. More than 650 different levansucrase sequences were studied and results were obtained such as phylogenetic trees and conserved residues that could be essential for biocatalysis machinery.

Authors want to acknowledge Spanish Ministry of Economy and Competitiveness for funding support (Project Code: CTQ2016-78988-R). Álvaro González-Garcinuño also acknowledges his PhD grant (F.P.U.) from the Spanish Ministry of Education, Culture and Sport (Ref. FPU14/04914).

FEMS7-2721
Biotechnology / Synthetic Biology / Systems Biology

DEVELOPMENT OF A MUCOSAL VACCINE AGAINST HIV BASED ON GENETICALLY-ENGINEERED SACCHAROMYCES CEREVISIAE PROBIOTIC STRAINS

B. Douradinha[1]
[1]*Fondazione RiMED, Medicina Rigenerativa e Tecnologie Biomediche, Palermo, Italy*

Backgrounds

A vaccine against HIV must not only elicit both cellular and humoral arms of the immune system, but also induce a mucosal immunity. Sexually transmitted HIV uses mucosal ports of entry, and a mucosal immune response would both prevent a new infection and its further spreading. Genetically engineered *Saccharomyces cerevisiae* strains expressing HIV antigens have shown promising pre-clinical results, as their can stimulate a T cell response but tend to induce a poor mucosal immune response.

Objectives

In this work, we propose the use of probiotic *S. cerevisiae* strains which have been genetically manipulated to express on their surface the HIV Gag antigen. Probiotic *S. cerevisiae* strains are known to naturally induce an immune response in the colon and to be resistant to the gastrointestinal harsh environments, such as acidic gastric juice and bile salts.

Methods

Probiotic *S. cerevisiae* strains were transformed with the bicistronic plasmid pCEV-G1-Km (pCEV) in its simple form or with the HIV *gag* gene (pJRP). We observed that this genetic modification did not impair neither phagocytosis by human dendritic cells (DCs) from healthy donors *in vitro* nor resistance to simulated gastrointestinal stresses. Based on the cell surface markers and cytokines secreted by healthy donors DCs following genetically engineered yeasts, we assume these immune cells polarize in a type 1 response. To measure a specific HIV Gag response, we matured DCs derived from an HIV+ patient with transformed yeasts and incubated them with autologous T cells from the same patient. Only DCs which have been in contact with pJRP-transformed probiotic *S. cerevisiae* strains were able to efficiently perform HIV Gag antigen presentation to T cells, as observed by clonal expansion of the former when later incubated with a Gag peptide pool.

Conclusions

Our results show that genetically engineered probiotic strains of *S. cerevisiae* are promising vaccination strategies against HIV.

FEMS7-2664
Biotechnology / Synthetic Biology / Systems Biology

ACTINOBACTERIAL GENOME MINING FOR NOVEL ANTIMICROBIAL PEPTIDE DISCOVERY

K. Durrell[1], M. Le Roes-Hill[1], L. Dicks[2]
[1]Institute of Biomedical and Microbial Biotechnology,
Biocatalysis and Technical Biology Research Group, Cape Town, South Africa
[2]Stellenbosch University- Faculty of Science, Microbiology, Cape Town, South Africa

Backgrounds

The emergence of new mechanisms of resistance among a number of organisms has decreased the effectiveness of current antimicrobial agents. The discovery of new classes of antimicrobial compounds based on targets identified from bacterial genomics is historically invaluable as a source of antimicrobial drugs. Genome sequencing has revealed that 35-40% of the biosynthetic genes in actinobacterial genomes encode for novel compounds. These organisms therefore represent a vast resource of novel bio-active compounds and will be exploited in this study.

Objectives

To analyse the genome sequences of selected actinobacterial strains for the presence of novel secondary metabolite biosynthetic gene clusters (smBGCs) and to preliminarily determine their potential to produce novel bio-active compounds.

Methods

The genome sequences of five actinobacterial strains were analysed for the presence of novel ribosomally synthesized and post-translationally modified peptide (RiPP) smBGCs using the online predictive tool antiSMASH. The strains were cultivated on a solid basal salts medium supplemented with various carbon sources for optimal antimicrobial production, bio-active compounds extracted, and the antimicrobial activities of the extracts were preliminarily determined.

Conclusions

It is predicted that the five actinobacterial strains have 8 to 55 smBGCs, of which at least 40% represent novel compounds. Extracts prepared from the strains exhibited activity against a methicillin-resistant *Staphylococcus aureus* ATCC 33591, *Mycobacterium aurum* A+ and *Candida albicans* ATCC 90028. In future, in order to access the full biosynthetic potential of the actinobacterial strains, standard drug discovery techniques and more modern genome-based coupled with culture-based drug discovery techniques will be performed in parallel.

FEMS7-1002
Biotechnology / Synthetic Biology / Systems Biology

EFFECT OF ADDITIVE LACTOBACILLUS PLANTARUM ON AMMONIUM PRODUCTION IN WHOLE PLANT CORN SILAGE DURING PROLONGED STORAGE

M. Duvnjak[1], *M. Čuklić*[1], *K. Gazić*[1], *J. Pintar*[1], *D. Grbeša*[1]
[1]*University of Zagreb Faculty of Agriculture, Department of Animal Nutrition, Zagreb, Croatia*

Backgrounds

Ensiling is a method for moist forage preservation based on natural fermentation of sugars to acids, mainly lactic acid. *Lactobacillus plantarum* is often used as additive during ensiling to promote optimal fermentation resulting in rapid lactic acid production and pH drop thus improving dominant nutrients preservation. Ammonium production is simple indicator of protein degradation and deamination in silages. Literature states proteolysis is active primarily during first few weeks of silage production. Excess proteolysis in silages is connected with multiple negative effects on silage quality and animal performance.

Objectives

In the present study, the application of *Lactobacillus plantarum* on ammonium production in WPCS during prolonged storage of silage was investigated.

Methods

Yellow corn hybrid (Bc 462) was ensiled in five replications in laboratory scale silos without and with *Lactobacillus plantarum* additive (300000 CFU/g fresh material). Silages were sampled at the beginning and on 15th, 48th, 98th, 182th, 274th and 364th day of ensiling when contents of ammonium (g/kg of CP), crude protein (CP) and DM were monitored. Effects of time, silage additive and their interactions on silages were tested using the PROC MIXED procedure in SAS 9.3.

Conclusions

The analyses showed that the ammonium contents increased in silages during whole period of prolonged storage ($P<0,05$) and were under influence of silage additive ($P<0,05$) and defined interactions ($P<0,05$). Control silages had higher ammonium contents, except on the 182th day when silages with *Lactobacillus plantarum* had higher values. We conclude that application of additive reduced negative deamination activity that was undoubtedly present during whole storage period.

FEMS7-0267
Biotechnology / Synthetic Biology / Systems Biology

RE-FACTORING PSEUDOMONAS PUTIDA FOR VALORIZATION OF LIGNOCELLULOSE-DERIVED SUGARS

P. Dvořák[1], V. de Lorenzo[1]
[1]Centro Nacional de Biotecnología CNB-CSIC, Systems and Synthetic Biology Program, Madrid, Spain

Backgrounds

Sugars and aromatic compounds derived from lignocellulosic waste can serve as cheap substrates for biotechnological production of value-added chemicals. However, well-defined microbial platforms that could efficiently perform such task are scarce. Pseudomonas putida KT2440 is a GRAS-certified, robust soil bacterium with versatile metabolism and high stress tolerance, a host well suited for biotechnological operations. P. putida KT2440 has been employed for production of fine chemicals from glucose and for processing lignin-derived aromatics. But its potential for valorization of other products of lignocellulose decomposition is limited due to the lack of metabolic traits.

Objectives

The objective of this study was to empower P. putida with novel biocatalytic functions that would pave the way towards its application in biotechnological recycling of lignocellulosic waste.

Methods

To meet this goal, we have adopted as a cellular chassis an in-house derivative of P. putida KT2440, strain P. putida EM42 with streamlined genome and superior physiological properties. The native and heterologous pathways for D-xylose utilization and valorization were successfully tested in our chassis. Functional screening of cellulases from distinct sources revealed enzymes enabling rapid growth of EM42 on products of cellulose decomposition and parallel accumulation of valuable biopolymers within the cell.

Conclusions

The obtention of a P. putida strain capable of efficient utilization of cellulose and hemicellulose-derived sugars for biomass formation and production of desirable chemicals provides a showcase of how rational orchestration of the metabolic properties of P. putida expands the catalytic scope of this bacterium towards industrial applications.

FEMS7-1613
Biotechnology / Synthetic Biology / Systems Biology

BIOACTIVE COMPOUNDS FROM CYANOBACTERIA

C. Edwards[1], *L. Lawton*[1]
[1]*Robert Gordon University, Pharmacy & Life Sciences, Aberdeen, United Kingdom*

Backgrounds

Algae are an essential component of global ecosystems using light and carbon dioxide to produce organic carbon and oxygen. Consequently they are found in diverse habitats including terrestrial and aquatic habitats, hot springs to Antarctic mats. The ecological diversity has resulted in chemical diversity and they increasingly investigated as sources of new chemical entities in drug discovery programs, in particular anti-cancer.

Objectives

Production of a wide array of peptides for research/commercial use.

Methods

Algae (400-500 L per month) were grown in parallel batch cultures. Cells were harvested and extracted using methanol, cleaned up by automated flash chromatography with final polish by preparative high performance liquid chromatography (HPLC). Throughout the process quality and quantity of compounds in extracts/fractions were tracked by ultra performance liquid chromatography – photodiode array-mass spectrometry (UPLC-PDA-MS) to ensure high purity is achieved. Due to the highly toxic nature of the compounds, extreme precautions were exercised throughout processing, with frequent review. In addition, packing and shipping strategies were developed as the peptides are classified as extremely dangerous according to transport regulations so the usual couriers such as FedEx will not take them. In addition, as they are considered as potential weapons of mass destruction, export licence and control is essential.

Conclusions

Despite all the challenges a wide array of bioactive peptides were produced underpinning research projects and providing revenue (£420 K GBP).

FEMS7-1372
Biotechnology / Synthetic Biology / Systems Biology

YEAST-BASED BIODIESEL PRODUCTION FROM INDUSTRIAL WASTES

K. Erkan Türkmen[1], E.D. Ulusman[1], N. Aksöz[1]
[1]Hacettepe University Faculty Of Science, Biology, Ankara, Turkey

Backgrounds

In recent years, alternative energy sources have been sought for fossil fuels. Biodiesel is a cheap and renewable alternative that has the most potential to advance. Yeasts are more preferred than other microorganisms due to their rapid growth and high amount of oil production. For these reasons, these microorganisms are remarkable candidate for biodiesel production.

Objectives

In this study, we aimed to produce biodiesel from yeasts that were isolated from soil. Besides, industrial wastes were used as growth medium to reduce the cost and reuse industrial wastes properly.

Methods

Microorganisms were isolated from various soil samples. Antibiotic(Gentamycin) was added into isolation medium to eliminate bacterial growth. Subsequently microorganisms were inoculated into several media to improve oil production. For separating oil from yeast, Folch Method was carried out on each yeast isolate. Converting biodiesel was provided by transesterification process. As a result of GC-MS analysis, species identification of the highest biodiesel potential isolates were determined by 18s rRNA analysis.

Conclusions

According to results, this study confirmed our opinion about yeast based biodiesel production. Production of yeast based biodiesel using industrial wastes were successfully managed. Therefore, Yeast-Based Biodiesel can be considered an alternative energy sources to fossil fuel and further way of recycling industrial wastes.

FEMS7-1856
Biotechnology / Synthetic Biology / Systems Biology

BACTEROIDES THETAIOTAOMICRON ENDO-LEVANASE IN ACTION: DEGRADATION OF BACTERIAL AND PLANT LEVANS

K. Ernits[1], *T. Visnapuu*[1], *K. Viigand*[1], *T. Alamäe*[1]
[1]*University of Tartu, Genetics, Tartu, Estonia*

Backgrounds

Gut microbiota plays a huge role in human well-being by participating in food digestion, energy harvest, mineral absorption and inhibition of pathogenic bacteria. Growth of beneficial (probiotic) bacteria in the gut can be specifically promoted by prebiotic food ingredients such as inulin and inulin-type fructooligosaccharides (β-2,1 linked fructans) that are fermented by bifidobacteria and lactobacilli.

Objectives

Novel prebiotics have attracted interest of food scientists. Our workgroup is focusing on synthesis of novel β-2,6 linked prebiotic fructans – polymeric levans and oligomeric levan-type fructooligosaccharides (FOS). Prebiotic potential of these substrates on fecal consortia will be further assayed.

Methods

1) Levan and levan-type FOS are synthesized from sucrose using a highly active and stable levansucrase Lsc3 of *Pseudomonas syringae*.

2) Levan is degraded into FOS using the endo-levanase of *Bacteroides thetaiotaomicron*.

Conclusions

Lsc3 produces from sucrose FOS and levan. Levan is easily precipitated from the media with ethanol. Purification of FOS is laborious as a huge amount of glucose arising from the reaction must be removed.

We have cloned, overexpressed, purified and characterized the endo-levanase BT1760 of human gut commensal *Bacteroides thetaiotaomicron*. We showed that BT1760 produces FOS from various levans, including those of very high molecular weight. Importantly, the BT1760 also cleaves a plant levan (from timothy), whereas plant inulin was not degraded. Compared to few other studied bacterial endo-levanases, the BT1760 is the most active and can be regarded as a feasible catalyst for the production of FOS from levans.

Acknowledgements

This work was financed by ERC grant GLTMR1050P.

FEMS7-1087
Biotechnology / Synthetic Biology / Systems Biology

BIOCHEMICAL AND EVOLUTIVE INSIGHTS OF OLEATO-DERIVED OXYLIPIN PRODUCTION IN PSEUDOMONAS AERUGINOSA

M. Estupiñán[1,2], *P. Diaz*[2], *M.À. Manresa*[1]
[1]*University of Barcelona, Biology- Health and Environment, Barcelona, Spain*
[2]*University of Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain*

Backgrounds

Pseudomonas aeruginosa displays the ability to convert oleic acid into a class of hydroxylated fatty acids known as oxylipins, whose biotechnological applications have extensively been studied, constituting important emulsifying agents in food and cosmetics industries, acting as antibacterial or antifungal substances, or as intermediate compounds for fine chemistry. A diol synthase (DS) activity is responsible for such a conversion, which proceeds through the dioxygenation of oleic acid to release hydroperoxide 10-H(P)OME ((10*S*)-hydroxy-(8*E*)-octadecenoic acid), followed by conversion of the hydroperoxide intermediate into 7,10-DiHOME ((7*S*,10*S*)-dihydroxy-(8*E*)-octadecenoic acid).

Objectives

Identification of the gene/s involved in DS activity for functional and phylogenetic characterization of the encoded enzymes.

Methods

Gene identification was performed through genome mining. Functional characterization of the enzymes was done by mutant complementation, site-directed mutagenesis, and expression analysis. Bioinformatic approaches were used for phylogenetic and structural studies.

Conclusions

The genes responsible for the two enzymatic activities were identified: a dioxygenase (10*S*-DOX) encoded by *PA2077* catalyses the first step of the reaction, whereas the diol-synthase (7,10-DS) encoded by *PA2078* converts the hydroperoxide into 7,10-DiHOME.
Both genes constitute an operon, although they preserve their individual activities when cloned separately in *E. coli*, indicating that no heterocomplex formation is required. No orthologues of these genes were found in other species, being unique for *P. aeruginosa*. *In silico* analysis suggest that the two enzymes were originated by a gene duplication event, constituting a separate cluster among cytochrome c peroxidases, being thus the first characterized members of a new subfamily of bacterial peroxidases designated as Fatty acid-di-heme Cytochrome c peroxidases (FadCcp).

FEMS7-1115
Biotechnology / Synthetic Biology / Systems Biology

ANCIENT VOLCANIC SOILS AS A SOURCE OF BACTERIAL THERMOPHILIC LIPASES

M. Estupiñán Romero[1,2], *C. Buruaga*[1], *J. Ribera*[1], *A. Fillat*[1], *J. Martínez*[1], *P. Diaz*[1]
[1]*University of Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain*
[2]*University of Barcelona, Health- Biology and Environment, Barcelona, Spain*

Backgrounds

Bio-inspired processes have a low environmental impact, reduce the amount of waste material and minimize costs. Thermophilic enzymes provide natural tools for application in biotechnological processes requiring high temperature conditions and insights into evolutionary mechanisms of the current biosphere selective pressure.

Objectives

Isolation and characterization of bacterial thermophilic enzymes from ancient volcanic soils.

Methods

Microorganisms of samples from laurisilva soils in El Hierro (Canary Islands) were isolated and various enzymatic activities at temperature ranges between 20 and 60°C were tested. Strain JR3 was selected as highly lipolytic and identified by 16S rDNA sequencing. Degenerated primers were designed and lipase LipJ was cloned and characterized using *p*-NP-derivative substrates. Evolutionary divergence of LipJ has been studied both, *in silico* and by site-directed mutagenesis.

Conclusions

The isolated JR3 strain showed high lipase activity at temperatures above 60°C, and was selected for identification and study. Strain JR3 was assigned to the genus *Bacillus*, showing a 98% identity with *Bacillus cereus*. Analysis of the extracellular lipolytic activity of JR3 showed high tolerance to temperatures even higher than 80°C, maintaining the highest levels of activity when tested at 100°C. However, analysis of activity of the cloned lipase (LipJ) showed optimum activity at 30°C. When the 3D model of the cloned lipase was obtained, the typical structure of thermophilic lipases was observed, suggesting that this enzyme derives most likely from a thermophilic ancestor. Site-directed mutagenesis over the recognized thermophilic regions expands LipJ activity to higher temperatures (80°C) and on longer chain substrates, confirming the hypothesis of evolutionary drift.

FEMS7-0287
Biotechnology / Synthetic Biology / Systems Biology

NEW TAILORING BIOSYNTHETIC PROTEINS OF POLYKETIDE ANTIBIOTIC AURICIN IN STREPTOMYCES AUREOFACIENS CCM 3239

L. Feckova[1], C. Bekeova[1], R. Novakova[1], E. Mingyar[1], D. Homerova[1], J. Kormanec[1]
[1]Institute of Molecular Biology Slovak Academy of Sciences, gene expression, Bratislava, Slovak Republic

Backgrounds

The soil bacteria of the genus *Streptomyces* produce various secondary metabolites including many medicinally important antibiotics. Aromatic polyketides comprise a large group of structurally diverse secondary metabolites, which are produced by type II PKSs, which comprise three essential subunits (KS, CLF, ACP) and further subunits (KR, ARO, CYC) involved in modification of the nascent polyketide chain. Various tailoring reactions catalyzed by diverse oxygenases, reductases, methylases, and glycosyltranferases produce a final bioactive polyketide [1]. We previously identified polyketide cluster *aur1* in *Streptomyces aureofaciens* CCM 3239 responsible for production of a unique angucycline antibiotic auricin. It is produced during a narrow time period following entry into stationary phase. This unusual phenomenon likely arises from a complex regulation of auricin biosynthesis [2].

Objectives

Characterization of tailoring biosynthetic genes involved in the biosynthesis of auricin.

Methods

Growth of *S. aureofaciens* CCM3239 and analysis of secondary metabolite production was done as described in [3]. Disruption of the genes in *S. aureofaciens* CCM3239 was done as described in [3]. Transcription was determined by S1-nuclease mapping using RNA isolated from different growth phases as described in [3].

Conclusions

1, We characterized an operon *sa13, sa12, sa11, sa10*, encoding genes involved in biosynthesis of auricin in *S. aureofaciens* CCM 3239.

2, We identified promoter directing expression of the *sa13, sa12, sa11, sa10* operon and found its differential dependence upon two auricin-specific positive regulators, Aur1PR3 and Aur1PR4.

Acknowledgements. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0410.

References.

1. Hertweck C, Luzhetskyy A, Rebets Y, Bechthold A (2007) *Nat Prod Rep* 24, 162-190

2. Kormanec J, Novakova R, Mingyar E, Feckova L (2014) *Appl. Microbiol. Biotechnol.* 98, 45-60.

3. Novakova R, Rehakova A, Kutas P, Feckova L, Kormanec J (2011) *Microbiology-SGM* 157**,** 1629-1639.

FEMS7-1911
Biotechnology / Synthetic Biology / Systems Biology

ISOLATION OF AN EFFICIENT BACTERIAL CELLULOSE PRODUCER

J. Fernández[1], *G. Morena*[1], *R. Reina*[1], *J. Pastor*[1], *J. Martinez*[1]
[1]*University of Barcelona, Department of Genetics- Microbiology and Statistics, Barcelona, Spain*

Backgrounds

Bacterial cellulose (BC) is produced by some microorganisms. BC displays exceptional physicochemical properties: high crystallinity, high tensile strength, high hydrophilicity, and biocompatibility. These unique properties enable many successful applications and make BC a multifunctional biomaterial. An issue that restrains commercial production and extended application of BC is the low yield of the producing strains. Strains with higher yields would reduce costs in mass production.

Objectives

The aim of this study was to isolate new strains with a high yield of bacterial cellulose production and to characterise the obtained cellulose.

Methods

Samples of commercial wine vinegar were screened to isolate cellulose producing strains. Hestrin Schramm broth (HS) was used as an enrichment and production media. After incubation of the bacteria in liquid culture in static conditions, a cellulosic pellicle is obtained on the air-liquid interface. Cellulose production yield was compared with that from *Komagataeibacter xylinus*, a well-studied bacterial cellulose synthesizer. The most productive isolate was further investigated.

Conclusions

The most productive isolate, JF2, was subjected to 16S rRNA sequence analysis and identified as a putative *Komagataeibacter obodiens* strain. JF2 showed a higher efficiency than the control strain. Growing on HS medium supplemented with mannitol, JF2 produced 200% more cellulose than *K.xylinus*. Cellulose production curves over time showed that JF2 reached the maximum production at 5 days, while *K.xylinus* needed 7 days. SEM analysis of the pellicles showed homogeneous structure of cellulose ribbons. These results suggest that JF2 has potential as bacterial cellulose producer with industrial application.

FEMS7-1859
Biotechnology / Synthetic Biology / Systems Biology

PHYTOHORMONES, BIOFILMS AND PIGMENTS IN LICHEN ASSOCIATED BACTERIA: POTENTIAL FUNCTIONAL ROLES AND BIOTECHNOLOGICAL APPLICATIONS

À. Figàs-Segura[1,2], *E. Barreno*[2,3], *E. G. Biosca*[1]
[1]*Universitat de València, Microbiología y Ecología, Burjassot, Spain*
[2]*Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Botánica, Paterna, Spain*
[3]*Universitat de València, Botánica, Burjassot, Spain*

Backgrounds

Recently, the concept of lichen symbiosis has been expanded to include bacterial communities as integral components. In fact, lichens are currently recognized as associations of multiple species in which bacterial communities could contribute with different roles, such as stimulating lichen thalli growth and/or protecting them by the production of biofilm and pigments. However, some of these activities attributed to these bacteria have been based on culture-independent methods or hardly studied, being also of biotechnological interest.

Objectives

The objective was to evaluate the ability of a collection of culturable bacteria isolated from the Mediterranean lichen *Ramalina farinacea* to produce phytohormones, biofilms and pigments.

Methods

Phytohormones production was evaluated through the detection of auxins and the enzyme ACC-deaminase, biofilm formation in microtiter plates and bacterial pigments on conventional culture media. Selected strains were identified by partial *16S rRNA* gene sequencing.

Conclusions

A high percentage of bacterial strains produced phytohormones, mostly the auxin IAA and the ACC-deaminase enzyme being detected in minor proportion. Biofilm production was observed in more than 90% of the strains and around 70% of them produced pigments, being mostly yellow, pink, orange and white. These activities could favor lichens colonization and adaptation to a wide variety of environmental conditions, by offering protection against biotic and abiotic factors and/or promoting growth within the lichen thallus. Further, their potential applications could be in agriculture and pharmaceutical and textile industries, among others. Finally, selected bacterial strains were ascribed to diverse taxa. (PROMETEOII/2013/021; VALi+d fellowship; UV-Research Support to BACPLANT).

FEMS7-2441
Biotechnology / Synthetic Biology / Systems Biology

EFFECTS OF ROTATING MAGNETIC FIELD EXPOSURE ON THE YIELD AND PROPERTIES OF BACTERIAL CELLULOSE SYNTHETIZED BY GLUCONACETOBACTER XYLINUS

K. Fijałkowski[1], R. Drozd[2], A. Żywicka[2], M. Kordas[3], M. Konopacki[3], A. Junka[4], P. Szymczyk[5], R. Rakoczy[3]
[1]West Pomeranian University of Technology- Faculty of Biotechnology and Animal Husbandry, Department of Immunology- Microbiology and Physiological Chemistry, Szczecin, Poland
[2]West Pomeranian University of Technology in Szczecin, Department of Immunology- Microbiology and Physiological Chemistry, Szczecin, Poland
[3]West Pomeranian University of Technology in Szczecin, Institute of Chemical Engineering and Environmental Protection Processes, Szczecin, Poland
[4]Medical University of Wroclaw, Department of Pharmaceutical Microbiology and Parasitology, Wrocław, Poland
[5]Wroclaw University of Technology, Centre for Advanced Manufacturing Technologies, Szczecin, Poland

Backgrounds

Bacterial cellulose (BC) is a natural polymer produced by bacteria *Gluconacetobacter xylinus*. The application of the magnetic field is a promising alternative to the conventional BC production methods.

Objectives

The main aim of the presented study was to evaluate the impact of rotating magnetic field (RMF) on BC synthetized by *G. xylinus* in regard to its production rate and quality parameters.

Methods

The cultures of *G. xylinus* were exposed to RMF of frequency equals 50 Hz and magnetic induction 34 mT for various time periods at different stages of *G. xylinus* cultivation, in the customized RMF exposure system. The obtained BC samples were analyzed using a set of analytical methods including ATR-FTIR, XRD, SEM, degree of polymerization, dry weight and water related properties assessment.

Conclusions

It was found, that the application of RMF, regardless of the exposure mode applied, significantly increased the cellulose thickness, weight and capacity of swelling in water in comparison to the unexposed controls. The changes of water-related properties were reflected by the degree of BC porosity and its fibril arrangement. Another significant observation, found in the synchronus 2D correlation of ATR-FTIR spectra was the impact of RMF on the dynamics of the formation of BC microfibers crystallinity. Therefore, it can be concluded, that the use of RMF may provide a novel technique for altering BC biogenesis and when fully developed, may find application in the multiple biotechnological applications.

This study was supported by the National Centre for Research and Development in Poland (Grant No. LIDER/011/221/L-5/13/NCBR/2014).

FEMS7-2495
Biotechnology / Synthetic Biology / Systems Biology

ASSOCIATION BETWEEN TYPE OF PURIFICATION, CYTOTOXICITY AND PRESENCE OF METABOLITES WITHIN CELLULOSE MEMBRANES PRODUCED BY GLUCANCETOBACTER XYLINUS STRAINS

K. Fijałkowski[1], A. Junka[2], A. Ząbek[3], K. Mikołajewicz[4], G. Chodaczek[4], P. Szymczyk[5], A. Żywicka[6], M. Dziadas[7], M. Bartoszewicz[8]
[1]West Pomeranian University of Technology- Faculty of Biotechnology and Animal Husbandry, Department of Immunology- Microbiology and Physiological Chemistry, Szczecin, Poland
[2]Wrocław Medical University, Department of Pharmaceutical Microbiology and Parasitology, Wroclaw, Poland
[3]Wroclaw University of Technology, Department of Bioorganic Chemistry, Wrocław, Poland
[4]Wroclaw Research Centre EIT+, Confocal Microscopy Laboratory, Wrocław, Poland
[5]Wroclaw University of Technology, Centre for Advanced Manufacturing Technologies CAMT/FPC, Wrocław, Poland
[6]West Pomeranian University of Technology in Szczecin, Department of Immunology- Microbiology and Physiological Chemistry, Szczecin, Poland
[7]Wrocław Medical University, Department of Bromatology and Dietetics, Wrocław, Poland
[8]Wrocław Medical University, Department of Pharmaceutical Microbiology and Parasitology, Wrocław, Poland

Backgrounds

Bacterial cellulose (BC) is a natural polymer produced by bacteria *Glucanacetobacter xylinus*. BC exhibits superior purity when compared with plant cellulose, so treatment is not required to remove unwanted polymers and contaminants (e.g. lignin, hemicellulose). However, BC obtained after fermentation always contains impurities like bacterial cells and culture media components. The application of BC (especially for medical purposes) requires removal of media residues and bacterial cells containing toxins like lipopolysaccharide.

Objectives

The study aimed at evaluation of various types of alkali rinsing with regard to their efficacy in terms of removal, not only of bacteria but also bacterial metabolites, from cellulose matrices formed by *G. xylinus* strains. Moreover, we tested the type of alkali rinsing on membrane cytotoxicity *in vitro* and we compared matrices' ability to induce oxidative stress in macrophages.

Methods

For the production of cellulose, three reference *G. xylinus* strains were used of. In order to remove bacterial cells and media components the BC samples were purified using 3 different concentrations of NaOH solution. For one group of BC samples, the purification procedure using a particular NaOH concentration was repeated two times and for another group, four times.

Conclusions

Our results indicated that the type of alkali rinsing should be adjusted to specific *G. xylinus* strains that are used as cellulose producers to obtain safe biomaterials in the context of low cytotoxicity and macrophage induction.

This study was supported by the National Centre for Research and Development in Poland (Grant No. LIDER/011/221/L-5/13/NCBR/2014).

FEMS7-2471
Biotechnology / Synthetic Biology / Systems Biology

SINGLE NUCLEOTIDE MUTATIONS LEAD TO IMPROVED FUNCTIONALITY OF SFGFP IN BIOTECHNOLOGICALLY IMPORTANT THERMOPHILIC CHASSIS

E. Frenzel[1], *R. van Kranenburg*[2], *O.P. Kuipers*[1]
[1]*University of Groningen, Department of Molecular Genetics, Groningen, The Netherlands*
[2]*Wageningen University, Laboratory of Microbiology, Wageningen, The Netherlands*

Backgrounds

Fluorescent reporter proteins (FP) have become an indispensable tool for the optimization of microbial cell factories and in synthetic biology *per se*. Their applicability is, however, constrained by species-dependent performance and misfolding at elevated temperatures.

Objectives

Flow cytometry-based benchmarking of a set of GFPs, sfGFPs and species-specific codon-optimized variants revealed that none of the proteins was satisfyingly detectable in *Parageobacillus thermoglucosidans*. This indicates that rational engineering does not necessarily lead to an optimized expression.

Methods

We employed an undirected mutagenesis approach coupled to fluorescence-activated cell sorting in order to isolate sfGFP variants active in the chassis background at 60°C.

Conclusions

Unexpectedly, a few nucleotide substitutions, including silent mutations, significantly improved the functionality in *P. thermoglucosidans*. This underpins the strong codon-dependence of FP performance, which might be connected to modulation of the translation and folding speed, especially at higher temperatures.

The novel sfGFP variants were active in other industrially relevant thermophilic spore formers, thus proving their broad applicability. The sfGFP variants were furthermore used as output elements to explore the transcriptomic landscape of *P. thermoglucosidans* grown under a broad set of conditions. This enabled us to study the architecture of promoters and regulons, as well as the heterogeneity of gene expression on the single-cell level.

Results from ongoing studies will be presented and hypotheses on the impact of single nucleotide mutations leading to synonymous amino acid substitutions and temperature stability of sfGFP will be discussed.

FEMS7-1220
Biotechnology / Synthetic Biology / Systems Biology

CHARACTERIZATION OF A NEW THERMOPHILIC B GLUCOSIDASE FROM DYCTIOGLOMUS TURGIDUM

F. Fusco[1], R. Ronca[1], G. Fiorentino[1], P. Contursi[1], S. Bartolucci[1], D. Limauro[1]
[1]University of Naples Federico II, Biology, Naples, Italy

Backgrounds

Biomass is an alternative source of renewable energy and can represent a new source for feedstock and chemical production; cellulose, the major component of biomass, is a linear polymer of glucose linked by β-1,4-glycosidic bonds. It is hydrolyzed to glucose by synergic action of endoglucanase, exoglucanase and b-glucosidase that complete the final step cleaving cellobiose in glucose. The improvement of enzymatic performance is strongly requested in bioprocesses; for this reason, thermozymes are often explored because they not only are stable at high temperature but are unusually resistant in organic solvents and detergents.

Objectives

This study is focused on the characterization of a new thermophilic b-glucosidase of the hyperthermophilic bacterium *Dyctioglomus turgidum*. Synthetic gene *Dtubglu* (Dtur_0462) from *D. turgidum* genome was constructed exploiting *Escherichia coli* codon usage. *Dtu*bGlu, expressed in *E. coli*, was purified and biochemically characterized.

Methods

Enzyme purification was performed by heat treatment and affinity chromatography on a His-Trap connected to an AKTA Explorer system (GE Healthcare); β-Glucosidase was assayed using p-nitrophenyl- β-Dglucopyranoside (pNPG) in microplate reader (Synergy H4, Biotek).

Conclusions

A new promising hyperthermophilic b-glucosidase, *Dtu*bGlu of *D. turgidum* was identified. Enzymatic activity was assayed on different substrates and kinetically characterized on pNPG showing a K_M value of 0.53 mM. The optimal pH (pH 5.4) and temperature (80°C) were determined. *Dtu*bGlu shows stability at high temperature (100% of activity after 3 h at 80°C). These features make this enzyme an excellent candidate for technological applications.

FEMS7-1912
Biotechnology / Synthetic Biology / Systems Biology

METAGENOMIC AND PROTEOMIC ANALYSIS OF LAB-SCALE, MESOPHILIC ANMBR AND THERMOPHILIC CSTR BIOGAS REACTORS RECEIVING MICROALGAL BIOMASS FEEDSTOCK FROM A PHOTOBIOREACTOR RECOVERING WASTEWATER EFFLUENT NUTRIENTS

J. Gaby[1], S. Greses-Huerta[2], M. Arntzen[1], D. Aguado[3], J. Ferrer[3], A. Seco[2], S. Horn[1]
[1]Norwegian University of Life Sciences NMBU,
Faculty of Chemistry- Biotechnology and Food Science IKBM, Ås, Norway
[2]Universty of Valencia, Department of Chemical Engineering, Valencia, Spain
[3]Polytechnic University of Valencia, Institute of Hydraulic and Environmental Engineering, Valencia, Spain

Backgrounds

Effluent from sewage treatment plants can contain high amounts of nitrogen and phosphorus which could eutrophicate water bodies. As an alternative to release or further costly treatment, wastewater treatment effluent may be used as a source of nutrients to grow *Scenedesmus* spp. microalgae in a membrane photobioreactor (MPBR), and the microalgal biomass may be subsequently fed to a biogas reactor.

Objectives

Recover genomes of taxa involved in the anaerobic digestion of microalgal biomass and examine the expression of pathways for cellulose, hemicellulose, lignin and protein hydrolysis.

Methods

Microalgal biomass was harvested from a MPBR as feedstock for a mesophilic (35°C) anaerobic membrane bioreactor (AnMBR) and a thermophilic (55 °C) continuous stirred tank reactor (CSTR) for production of biogas. We studied the reactor and feedstock microbial communities through 16S amplicon sequencing, shotgun metagenomics, and metaproteomics.

Conclusions

From 47 gigabases of shotgun metagenomic data we recovered 313 genome bins including 13 that were >95% complete. In the thermophilic CSTR, *Coprothermobacter*, a potential protein degrader, and the candidatus EM3 phylum comprised approximately 1/3 each of the microbial community. We are using metagenomics to infer the characteristics that allow EM3 bacteria to decompose microalgal biomass. The mesophilic AnMBR community was more diverse and included the dominant taxa W22 (20%), a sugar fermenter, and T78 (15%), a carbohydrate and lignocellulosic degrader. Through proteomics analysis, we determined the abundance of 2936 proteins of which only 17% had hypothetical gene annotations. The most abundant proteins encoded diverse cellular processes including methanogenesis and nutrient acquisition.

FEMS7-2547
Biotechnology / Synthetic Biology / Systems Biology

AN ESCHERICHIA COLI STRAIN REFERENCE PANEL FOR GENOTYPE-TO-PHENOTYPE ANALYSIS

M. Galardini[1], C.L. Herrera[2], A. Koumoutsi[2], J.A. Cordero Varela[1], A. Telzerow[2], A. Typas[2], P. Beltrao[1]
[1]EMBL-EBI, EBI, Cambridge, United Kingdom
[2]EMBL, Genome Biology, Heidelberg, Germany

Backgrounds

Understanding how genetic variants, across individuals of the same species, contribute to variability in phenotypes is a central question in genetics. However, we currently lack models that can describe how genetic variability impacts on different molecular layers (e.g. protein stability and function) and how the ensemble of these effects manifests to cellular phenotypes.

Objectives

.

Methods

To address this problem, we collected a panel of ~1000 *Escherichia coli* strains (natural isolates and evolved strains) that were genotyped and phenotyped across ~200 chemical and environmental conditions in order to develop and test predictive phenotypic models. Molecular and cellular knowledge of *E. coli* K12 was used to model the consequence of mutations across a subset of ~730 fully sequenced strains.

Conclusions

We observed that deleterious mutations in conditionally important genes in *E. coli* K12 are often but not always associated with poor growth in the respective condition in other strains. This suggests that conditional essentiality is not strictly conserved across strains. We also observed that both non-synonymous substitutions and gene content variability have the same importance in determining the phenotype, highlighting the importance of recombination in bacteria.
Collectively, our dataset of genotypes and phenotypes constitutes a resource for the development of predictive phenotypic models and highlights the evolutionary plasticity of conditional essentiality in *E. coli*. We anticipate that this strain reference panel will become a community resource to tackle the various facets of the genotype-to-phenotype problem.

FEMS7-1723
Biotechnology / Synthetic Biology / Systems Biology

IMPROVED PRODUCTION OF THE ANTIBIOTIC ACTINORHODIN IN STREPTOMYCES COELICOLOR IMMOBILIZED-MYCELIAL CELL CULTIVATIONS

A. Sutera[1], F. Lopresti[2], R. Dolcemascolo[1], R.M. Fontana[1], L. Botta[2], A.M. Puglia[1], G. Gallo[1], R. Scaffaro[2]
[1]University of Palermo, STEBICEF, Palermo, Italy
[2]University of Palermo, DICAM, Palermo, Italy

Backgrounds

Actinomycetes, Gram-positive filamentous bacteria, are the most prolific source of industrially important bioproducts, like antibiotics and enzymes. *Streptomyces coelicolor*, producing different antibiotics including the blue pigmented actinorhodin (ACT), is a model organism for antibiotic production. During actinomycete liquid cultivations, one of the major causes negatively affecting bioproductivity is the growth of a pellet-shaped biomass.

Objectives

i) Evaluation of ACT production in *Streptomyces coelicolor* M145 mycelial cells immobilized on polycaprolactone (PCL) and polylactic acid (PLA) nanofiber membranes, modified or not by an O_2-plasma treatment.
ii) Identification of gene products associated with the improvement of ACT production.

Methods

- Scanning electron microscope (SEM) analysis.
- Determination of total protein content.
- Determination of ACT amount.
- Differential proteomic analysis based on 2-D Fluorescence Difference Gel Electrophoresis (2D-DIGE) and mass spectrometry (MS) procedures.

Conclusions

Starting from spore suspensions, *S. coelicolor* immobilized-mycelial cells were obtained in liquid cultivations using modified or unmodified PCL and PLA membranes. All the immobilized-mycelial cell cultivations showed an increment of ACT yield in comparison to free-mycelial cells, with O_2-plasma treated PLA membranes the most effective ones. Differential protein patterns of immobilized- and free-cell cultivations were revealed by a preliminary SDS-PAGE analysis. Therefore, a differential proteome analysis, based on 2D-DIGE and MS procedures, is being carried out to highlight molecular processes and metabolic pathways differentially regulated in immobilized- and free-cell cultivations.

FEMS7-2028
Biotechnology / Synthetic Biology / Systems Biology

UNRAVELLING THE SECRETOME OF THE PLANT PATHOGEN ALTERNARIA: NEW BIOTECHNOLOGICAL TOOLS IN WOODWORKING AND LIGNOCELLULOSIC RELATED INDUSTRIES

L. García-Calvo[1], R.V. Ullán[2], M. Fernández-Aguado[1], A.M. García-Lino[3], A. Sola-Landa[1], K. Kosalková[4], C. Barreiro[5,6]
[1]INBIOTEC- Instituto de Biotecnología de León, Environment Department, León, Spain
[2]mAbxience, Upstream Production, León, Spain
[3]Área de Fisiología, Departamento de Ciencias Biomédicas, León, Spain
[4]INBIOTEC- Instituto de Biotecnología de León, Biopharmacy and Biomedicine Department, León, Spain
[5]INBIOTEC- Instituto de Biotecnología de León, Proteomics, León, Spain
[6]Área de Microbiología, Departamento de Biología Molecular, León, Spain

Backgrounds

The woodworking industry is an essential economic sector which encompasses over 280,000 companies in the European Union. In this context, wood decay processes are of prominent interest, considering both their industrial applications in biorefinery and their importance in the prevention of wood degradation.

Fungi are known as active degraders of lignocellulosic materials in nature.

Objectives

Alternaria sp. is a fungal genus which includes endophytic, saprobic and plant pathogenic species. The aim of this study was to analyse the secretome from *Alternaria tenuissima*, given its potential as a phytopathogenic mold able to colonise and survive on wood.

Methods

The *Alternaria tenuissima* strain used in the studies was grown in presence of different lignocellulosic substrates and the enzymatic extract secreted to the culture medium was obtained at several time points. Two-dimensional gel electrophoresis of proteins was performed and proteins were subsequently identified by means of a MALDI-TOF/TOF mass spectrophotometer.

Conclusions

The analysis of the results showed a total of 80 identified proteins, 37 of which were isoforms. Out of the total number of 80, 33 proteins were successfully assigned to a KOG functional category (*Eukaryotic Clusters of Orthologous Groups,* contained in the COG database). 76% of the determined proteins belonged to the G category (proteins related with carbohydrate metabolism and transport) and, the remaining, to categories K, O and M.

These results are congruent with the expected secretome in the analysed conditions, and they confirm *Alternaria tenuissima* as an interesting species in the wood decay processes.

FEMS7-2066
Biotechnology / Synthetic Biology / Systems Biology

PROTEOME RESPONSE OF THE PENICILLIN-PRODUCING MOLD PENICILLIUM CHRYSOGENUM TO CASEIN PHOSPHOPEPTIDES AND CACL2

R. Domínguez-Santos[1,2], K. Kosalková[1], C. Barreiro[2,3], L. García-Calvo[4], J.F. Martín[2], A. Ibáñez[5], A. Morales[3], C. García-Estrada[1]
[1]INBIOTEC- Instituto de Biotecnología de León, Biopharmacy and Biomedicine Department, León, Spain
[2]Área de Microbiología, Departamento de Biología Molecular, León, Spain
[3]INBIOTEC- Instituto de Biotecnología de León, Proteomics, León, Spain
[4]INBIOTEC- Instituto de Biotecnología de León, Environment Department, León, Spain
[5]Instituto de Investigación de la Viña y el Vino, Universidad de León, León, Spain

Backgrounds

Penicillin biosynthesis in *Penicillium chrysogenum* is one of the best characterized secondary metabolism processes. However, the mechanism by which penicillin is secreted still remains to be elucidated. Calcium (Ca^{2+}) plays an important role in the metabolism of filamentous fungi, and casein phosphopeptides (CPP) are involved in Ca^{2+} internalization.

Objectives

Taking into account the role played by Ca^{2+} and CPP in the secretory pathway and considering the positive effect that Ca^{2+} exerts on penicillin production, the analysis of global protein changes produced after CPP/$CaCl_2$ addition is very helpful to decipher the processes related to the biosynthesis and secretion of penicillin.

Methods

A wide proteome comparative analysis using 2D-DIGE was carried out to characterize the mechanisms triggered by CPP/$CaCl_2$.

Conclusions

The most interesting proteins that were overrepresented after CPP/$CaCl_2$ addition were a peroxisomal catalase, three proteins of the methylcitrate cycle, two aminotransferases and cystationine β-synthase, which are directly or indirectly related to the formation of penicillin amino acid precursors. Importantly, two of the enzymes of the penicillin pathway (isopenicillin N synthase and isopenicillin N acyltransferase) are clearly induced after CPP/$CaCl_2$ addition. Most of these overrepresented proteins are either authentic peroxisomal proteins or microbody-associated proteins. This evidence suggests that addition of CPP/$CaCl_2$ promotes the formation of penicillin precursors and the penicillin biosynthetic enzymes and stimulates the formation of peroxisomes and microbodies, which may be involved in transport of penicillin biosynthetic intermediates and finally in penicillin secretion.

FEMS7-1436
Biotechnology / Synthetic Biology / Systems Biology

IMPACT OF DIETARY NITROGEN DEPRIVATION ON BLATTABACTERIUM ENDOSYMBIONT AND HINDGUT MICROBIOTA IN BLATTELLA GERMANICA

C. García-Ferris[1], R. Domínguez[1], B. Pérez-Rocher[1], J.A. Castro[2], J. Baixeras[3], A. Moya[1], A. Latorre[1]
[1]Institute for Integrative Systems Biology I2SysBio, University of Valencia, Paterna, Spain
[2]Department of Biology- Faculty of Science, University of the Balearic Islands, Palma, Spain
[3]Cavanilles Institute of Biodiversity and Evolutionary Biology ICBiBE, University of Valencia, Paterna, Spain

Backgrounds

German cockroach, *Blattella germanica*, harbors two levels of symbiotic interactions. One is set around a single bacterial endosymbiont, *Blattabacterium*, located inside specialized fat body cells called bacteriocytes. Another, more complex, is the consequence of a highly diverse bacterial microbiota, inhabiting the luminal surface of the hindgut. This is one of the few known examples of an insect that keeps both gut microbiota and an endosymbiont. *Blattabacterium* plays a central role in the host nitrogen metabolism allowing the mobilization and recycling of urate deposits, in which excess of dietary nitrogen is stored, by contributing the urease activity that degrades urea to ammonia which is then recycled by the host into glutamine, needed for essential amino acids biosynthesis by *Blattabacterium*. In contrast, the function of the bacterial microbiome located in the *B. germanica* hindgut is presently far from being completely understood.

Objectives

The main objective is to assess whether the microbiome also collaborates in the host nitrogen metabolism once urate deposits depleted.

Methods

B. germanica adults were fed with an artificial diet free of N-protein to trigger urate mobilization and, after 9 weeks of treatment, fat body and hindgut were dissected. *Blattabacterium* and bacteriocyte populations were followed using QPCR and microscopy of fat body, and the bacterial composition of hindgut microbiome was determined using a metagenomic analysis.

Conclusions

We have evaluated whether a nitrogen-deprived diet induces changes in diazotrophic bacteria in the microbiota that could fix nitrogen supplying organically bound nitrogen to the host, thus complementing *Blattabacterium* in the nitrogen metabolism of the insect.

FEMS7-2090
Biotechnology / Synthetic Biology / Systems Biology

BIOTECHNOLOGIC POTENTIAL OF BACTERIAL STRAINS ISOLATED FROM A FOREST PEST (BARK BEETLES)

A. Fabryová[1], M. Kostovčík[1], A. Díez-Méndez[2], A. Jiménez-Gómez[2], L. Celador-Lera[2], Z. Saati-Santamaría[2], E. Menéndez[3], M. Kolařik[1], P. García-Fraile[1]
[1]Institute of Microbiology, Laboratory of fungal genetics and metabolism, Prague, Czech Republic
[2]University of Salamanca, Microbiology and Genetics Department, Salamanca, Spain
[3]University of Evora, Institute of Mediterranean Agricultural and Environmental Sciences ICAAM, Evora, Portugal

Backgrounds

Bark beetles establish symbiosis with several microbial strains which play different roles within the beetle holobiont, aiding for instance the beetle to hydrolyze the tree host with the production of hydrolytic enzymes to degrade the ingested bark and wood.

Objectives

In the present study, we aimed the isolation of bark beetle bacterial endosymbionts producing lignocellulolytic enzymes, as cellulases, xylanases and laccases, with potential applications in production of biofuels from biomass and capability for colorant degradation, with potential applications in the textile industry and for wastewater treatment.

Methods

We isolated and identified several bacterial strains from bark beetle species *Cryphalus piceae, Ips typographus* and *Pithophtorus pithophtorus,* collected in the Czech Republic from pine, spruces and fir trees, respectively. Strains were classified according to their 16S sequence as belonging to the genera *Erwinia*, *Pantoea*, *Curtobacterium*, *Yersinia*, *Pseudomonas, Staphylococcus* and *Rahnella*. Several plate screening methods were applied in order to detect lignocellulolytic enzymes and colorant degradation. Moreover, genome sequence of selected strains was obtained and analyzed looking for cellulases, xylanases, amylases and laccases.

Conclusions

Those strains are capable of colorants and plant cell compounds degradation. All isolates of the study showed capability of hydrolyzing several of the tested compounds. Besides, genome sequences revealed the presence of enzymatic machinery required for biomass hydrolysis

FEMS7-2174
Biotechnology / Synthetic Biology / Systems Biology

GENOME ANALYSIS BACTERIAL STRAINS ISOLATED FROM BARK BEETLES REVEALS THE PRESENCE OF GENES INVOLVED IN THE BIOSYNTHESIS OF ANTIBIOTICS AND OTHER MICROBIAL SECONDARY METABOLITES

P. García-Fraile[1], M. Kostovčík[1], Z. Saati-Santamaría[2], R. Martínez-García[2], I. Chapado-Pinto[2], R. Rivas[2], M. Kolařik[1]
[1]Academy of Science of the Czech Republic, Institute of Microbiology, Prague, Czech Republic
[2]University of Salamanca, Microbiology and Genetics, Salamanca, Spain

Backgrounds

Bark beetles establish symbiosis with several microbial strains which play different roles within the beetle holobiont. It has been described how some of these bacterial associates protect the bark beetle holobiont by producing substances which inhibit the growth of bark beetles' antagonists and therefore, those strains can be of great interest in pharmaceutical industry.

Objectives

The aim of this work was the detection of the genetic machinery for the synthesis of bioactive secondary metabolites with potential interest in biomedicine within the genome sequences of bacterial isolates from several bark beetle species.

Methods

Thus, we isolated bacterial associates from Ips cembre, Xylocleptes bispinus, Ips acuminatus and Pytogenes bidentatus adults and from Ips typographus larvae. The insect individuals had been collected from their tree hosts in different locations within the Czech Republic. Then, we tested the allelopathic interactions between our isolates and different pathogenic bacterial and fungal species. Those strains with greater capability to inhibit the pathogenic ones were selected to obtain their genome sequence, which was analyzed in order to find the genes potentially implicated in the synthesis of bioactive secondary metabolites.

Conclusions

Bioinformatic analysis of the genome sequence of bark beetles' bacterial associates revealed the capability of those strains to synthesize bioactive compounds with potential uses in pharmaceutical and biomedical industries.

FEMS7-1236
Biotechnology / Synthetic Biology / Systems Biology

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF SACCHAROMYCES STRAINS FROM DIFFERENT ENVIRONMENTAL NICHES AS A FUNCTION OF THE TEMPERATURE

E. García-Ríos[1], *A. Querol*[1], *J.M. Guillamón*[1]
[1]*Instituto de Agroquímica y Tecnología de los Alimentos, Biotecnología, Paterna, Spain*

Backgrounds

Production of useful products through microbial fermentation is a core activity of industrial biotechnology, and yeasts are one of the most widely used microorganisms in industrial biotechnology. This industry spends a huge amount of energy cooling or heating these processes, to fine-tune temperature as close as possible to the optimum growth temperature of the fermentative strains. The adaptation and tolerance of yeast strains towards temperatures outside the optimum range is crucial for economic and eco-efficient production processes for new and traditional fermentations.

Objectives

We evaluated the growth capacity of a collection of *Saccharomyces* strains belonging to different environments in order to improve some of these strains in terms of thermotolerance by using different genetic and metabolic engineering techniques.

Methods

To carry out this objective, experimental evolution and inter- and intraspecific hybridization experiments were performed to generated non-GMO improved strains. A multi-omic approach was also performed in strains with divergent phenotype at low and high temperatures. The better knowledge about yeast thermotolerance, provided by this systematic global approach, will be used to design new strategies of adaptive evolution.

Conclusions

Sulfur assimilation, nitrogen uptake, maintenance of the membrane asymmetry and oxidative stress response proved to be crucial mechanisms for the better thermotolerant capacity and they have been deeply analyzed by using different biochemical approaches.

FEMS7-1282
Biotechnology / Synthetic Biology / Systems Biology

GENOME-SCALE METABOLIC RECONSTRUCTION AND ANALYSIS OF SPHINGOPYXIS GRANULI STRAIN TFA

I. García-Romero[1], J. Nogales[2], E. Santero[1], B. Floriano[1]
[1]Andalusian Center for Developmental Biology,
Pablo de Olavide University and Department of Molecular Biology and Biochemistry Engineering, Sevilla, Spain
[2]Centro de Investigaciones Biológicas CIB, 2Environmental Biology Department, Madrid, Spain

Backgrounds

Sphingopyxis granuli strain TFA is a Gram-negative bacterium belonging to *Sphingomonadaceae* family whose most studied metabolic feature is its ability to use the aromatic organic solvent tetralin as the sole carbon and energy source. Regarding other metabolism aspects, this bacterium is able to accumulate poly-hydroxybutyrate (PHB) as carbon reservoir and, recently, it has been described as the first *Sphyngopyxis* representative able of growing anaerobically using nitrate as final electron acceptor. As several oligotrophic bacteria have been assigned to this genus, it is interesting to study other features of its metabolism that remain poorly or not characterized at all.

Objectives

The main objective of this work is to know better and deeply the TFA metabolic capabilities through the construction of a metabolic model at a genome scale.

Methods

Initially, two models of TFA were constructed based on *Escherichia coli* and *Pseudomonas putida* models, using the MrBac server. They were merged to obtain the first draft in which each reaction was manually reviewed. In order to complete the model, missing reactions were incorporated to fill the gaps in metabolic pathways thanks to the TFA genome annotation and metabolic/biochemical databases such as KEGG, BRENDA, MetaCyc and BiGG or by similarity search in Uniprot/SwissProt and the *Pseudomonas* Database.

Conclusions

The constructed model of TFA metabolism, *i*IG738, consist of 738 genes, 1392 reactions and 1107 metabolites classified in 95 metabolic subsystems. The reconstruction has highlighted diverse new carbon sources that TFA can use, which have been validated *in vivo*. Overall, *i*IG738 constitutes a powefull computational tool to evaluate, at system level, the metabolic traits of the oligotrophic bacterium TFA under a large array of environmental conditions.

FEMS7-2838
Biotechnology / Synthetic Biology / Systems Biology

CLONING AND EXPRESSION OF SCFV ANTIBODY FRAGMENT AGAINST PCRV PROTEIN OF PSEUDOMONAS AERUGINOSA IN E. COLI

E. Bayat[1], S. Hosseini[2], F. Barkhordari[2], R. Moazzami[2], H. Jahandar[3], F. Davami[2], L. Nematollahi[2], Y. Garoosi[2]
[1]Pharmaceutical Sceinces Branch- Islamic Azad University,
Faculty of Advanced Sceinces & Technology- Department of Molecular and cellular Sceinces, Tehran, Iran
[2]Pasteur Institute of Iran, Biotechnology Research Center, Tehran, Iran
[3]Pharmaceutical Sceinces Branch- Islamic Azad University,
Faculty of Advanced Sceinces & Technology- Department of Biotechnology, Tehran, Iran

Backgrounds

Pseudomonas aeruginosa is a major cause of hospital-acquired infections, particularly in mechanically ventilated patients. It is also a leading cause of death in cystic fibrosis patients. A key virulence factor associated with the disease severity of *P. aeruginosa* is its type III secretion system (T3SS) which injects bacterial toxins directly into the cytoplasm of host cells. PcrV is an important structural protein of the TTSS. An engineered human antibody Fab fragment that binds to the *P. aeruginosa* PcrV protein with high affinity has been identified which has potent in vitro neutralization activity against the TTSS.

Objectives

In this study an antibody molecule in the form of recombinant single chain variable fragment antibody (scFv) has been designed against PcrV protein in order to be expressed in *Escherichia coli* expressing strain, BL21.

Methods

cDNA molecule encoding the antibody fragment was cloned in pET28a expression vector. Cloning procedure was confirmed by sequencing and restriction digestion analysis. The expression vector was transformed into *E. coli* cells and the expression of recombinant protein was then induced by IPTG (0.5mM). SDS-PAGE was used to detect the expression of the desired protein. Western blotting using anti His-6 antibody was performed to confirm the expressed recombinant protein.

A 1175bp DNA fragment encoding the designed scFv antibody was cloned in pET28a plasmid and restriction digestion with NcoI/XhoI enzymes confirmed the gene. SDS-PAGE and western blot analysis confirmed presence of an induced 43kDa protein.

Conclusions

The results showed that E. coli BL21 can be a suitable strain for high level expression of anti PcrV-scFv protein. Purification, refolding and in vitro assays will be performed in next steps.

FEMS7-2017
Biotechnology / Synthetic Biology / Systems Biology

FROM DETECTION TO ACTION: PRODUCTION AND CHARACTERIZATION OF THE HIGHLY ACTIVE ANTIFUNGAL PROTEIN AFPB FROM THE CITRUS POSTHARVEST PATHOGEN PENICILLIUM DIGITATUM

S. Garrigues[1], *M. Gandía*[1], *F. Marx*[2], *J.F. Marcos*[1], *P. Manzanares*[1]
[1]*Instituto de Agroquímica y Tecnología de Alimentos IATA - CSIC, Department of Food Biotechnology, Paterna, Spain*
[2]*Medical University of Innsbruck, Division of Molecular Biology, Innsbruck, Austria*

Backgrounds

Antifungal proteins (AFPs) are small, cationic, cysteine-rich proteins that are secreted in large amounts by filamentous ascomycetes. Phylogenetic analyses suggested the grouping of AFPs in classes A, B and C. AFPs from class A have been widely studied, including the protein PAF from *Penicillium chrysogenum.* Previous attempts to detect the unique AfpB in the citrus postharvest pathogen *Penicillium digitatum* were not successful, despite high gene expression. PAF and AfpB show limited sequence identity.

Objectives

To use a *P. chrysogenum*-based expression system to produce and purify AfpB, and subsequently characterize its antifungal activity and properties.

Methods

A *P. chrysogenum*-based expression cassette consisting of *paf* 5´and 3´regulatory regions was used to direct the *afpB* gene expression in *P. digitatum*. AfpB was purified using cation-exchange chromatography. Antimicrobial assays were performed in 96-well plates.

Conclusions

The use of the *paf* gene promoter and terminator resulted in the efficient production and secretion of AfpB by *P. digitatum*, with no deleterious effect on the growth or virulence of the fungus, and the subsequent purification in high yields. AfpB has extreme thermal stability and resistance against proteolysis. AfpB showed potent antifungal activity against a range of filamentous fungi, with completely inhibitory concentrations around one micromolar, and was surprisingly active against *P. digitatum*. The AfpB structure was modeled and used to design synthetic peptides that allowed the mapping of fungal domains in the protein. AfpB is a very promising biomolecule for the development of novel antifungal agents.

FEMS7-1495
Biotechnology / Synthetic Biology / Systems Biology

COMPARATIVE GENOMICS TO DETERMINE PROBIOTIC PROPERTIES OF ENTEROCOCCUS FAECIUM 17OM39

V. Ghattargi[1], M. Gaikwad[1], S. Pandit[1], Y. Nimonkar[1], B. Meti[2], Y. Shouche[1], O. prakesh[1], D. Dhotre[1], S. pawar[1]
[1]National Centre for Cell Science, Microbial Culture Collection, pune, India
[2]Basaveshwar Engineering College, biotechnology, bagalkot, India

Backgrounds

The *Enterococcus faecium* 17OM39 was isolated from the gut of healthy Indian and an integrated approach of experimental evidence and genomic analysis was used to know its potential as probiotic. For numerous questions, the safety of probiotic strains should be carefully evaluated. It is therefore suggested that when considering an *Enterococcus* strain for use as a starter or probiotic culture, it is imperative that each particular strain should be carefully evaluated for the presence of all known virulence factors and antibiotic resistance.

Objectives

Whole genome comparison of Enterococcus faecium 17OM39 with other probiotic as well as pathogenic and non-pathogenic strains of same species

Knowing Pan, Core genome and Unique features

Identifying genes imparting probiotic properties, virulence factors and antibiotic resistance

Genome differences based on ecological niche

Methods

Whole genomes of ten strains were downloaded from the NCBI genomes. Bacterial pan genome annotations pipeline other bioinformatic tools are used to find the pan, core and unique genes. A list of fifty-three genes responsible for probiotic properties were screened within the genomes by performing BLASTX. The Comprehensive Antibiotic Resistance and Virulence Factors of Bacterial Pathogens databases were used to find the antibiotic resistance and virulence genes associated within the genomes.

Conclusions

Comparing the genomes of different strains of *Enterococcus faecium* isolated from a different source with our stain has improve the understanding of location and function of genes imparting probiotic features, antibiotic resistance, virulence and mobile elements. These insights have also help in understanding the genome differences based on ecological niche and the commensal and inherent probiotic properties of *Enterococcus faecium* 17OM39.

FEMS7-1512
Biotechnology / Synthetic Biology / Systems Biology

EMPLOYING COMPUTATIONAL APPROACH FOR SELECTION OF A POTENTIAL PROBIOTIC CANDIDATE

V. Ghattargi[1], M. Gaikwad[1], S. Pandit[1], Y. Shouche[1], B. Meti[2], D. Davaray[1], D. Dhotre[1], S. Pawar[3]
[1]National Centre for Cell Science, Microbial Culture Collection, pune, India
[2]Basaveshwar Engineering College, Biotechnology, Bagalkot, India
[3]NCCS, Microbial Culture Collection, pune, India

Backgrounds

The conventional methods for performing *in-vitro* cell culture-based assays and experiments are used by researchers to discover probiotic candidate. Computational genome analysis can be a cost-effective solution for the primary screening of potential probiotic candidate.

Objectives

Knowing the signature genes present in the genome of probiotic organisms

Methods

Ten bacterial genera containing species commonly used as probiotics for human consumption were compared based on publicly available complete genome sequences. The analysis included complete genome of *Lactobacillus* (93), *Lactococcus* (17), *Bifidobacterium* (65), *Leuconostoc* (15), *Saccharomyces* (2) as well as a selection of *Bacillus* (93), *Enterococcus* (28) *Streptococcus* (25), *Clostridium* (10) and *Escherichia* (21) genomes. The latter five genera included genomes from probiotic, commensal as well as pathogenic organisms to investigate if their non-pathogenic members shared more genes with the other probiotic genomes. List of known genes associated with probiotic properties, antibiotic resistance and virulence were screened from the literature and searched in all the genomes. Bioinformatic analysis was carried out using various available softwares and *in house* PERL and R scripts.

Conclusions

This study illustrates how over 369 genomes and 220 plasmids can be broadly compared using simple bioinformatic tools, and gain insights into the signature genes present in genomes of a probiotic candidate. These signature genes can be used for bio-prospecting of novel probiotic bacteria. We also believe the data presented here can assist in understanding the commensal and probiotic relationship of bacteria with their human host.

FEMS7-2808
Biotechnology / Synthetic Biology / Systems Biology - Part II

RE-ENGINEERING SLOW SAND FILTRATION AFFECTS THE TOTAL EUKARYOTIC COMMUNITY, BUT A KEYSTONE FUNCTIONAL FOOD-WEB IS RESPONSIBLE FOR PATHOGEN REMOVAL ACROSS ALTERNATIVE CONFIGURATIONS

J. Gibbs[1], *E. Porca Belio*[1], *G. Collins*[1]
[1]*National University of Ireland- Galway, Microbiology, Galway, Ireland*

Backgrounds

Slow Sand Filtration (SSF) is a low-energy, low-technology, chemical-free, simple-to-use approach for tertiary municipal wastewater treatment. The biologically-driven removal of potentially pathogenic bacteria is a key aspect of this technology, making it a suitable option when the treated effluent is being released into recreational, aquacultural or irrigation waterbodies.

Previous SSF studies have focused on the structure, and microbiology, of the bacterial community whilst the roles of eukaryotic microorganisms have been overlooked.

Objectives

Two distinct – a (i) traditional and (ii) modified, back-washed – SSF configurations were investigated at laboratory-scale to determine the composition of the microbial community in spatio-temporal biofilm samples.

Methods

Q-PCR, T-RFLP gene fingerprinting, and high-throughput sequencing of 16S and 18S rRNA genes were collectively deployed to characterise the total microbial community of the SSFs. A targeted approach based on DNA-stable isotope probing and rRNA sequencing was used to trace the functional community implicated in ^{13}C-labelled pathogenic *E. coli* retention in the differently-configured SSFs.

Conclusions

Depth (position in the SSF sand bed) and the configuration design more significantly affected changes to the eukaryotic community structure, diversity and species richness than to the bacterial community. Eukaryotes were also stronger, and more frequent, indicator organisms of configuration and depth. Tracing ^{13}C-labelled *E. coli* revealed a diverse web of eukaryotic bacteriovores implicated in pathogen retention and inactivation by the SSFs. Species responsible for greatest up-take of labelled biomass across the time-series used – namely Cercozoans, Oligohymenophorea (and other Cilliates) and Variosea amoebozoans – were identified as the predominant predators across both SSF configurations.

FEMS7-2793
Biotechnology / Synthetic Biology / Systems Biology - Part II

LOOKING FOR A FLEXIBLE BIOCATALYST: THE ROLE OF THE ACTIVE POCKET IN THE PRODUCT SPECIFICITY OF THE B-FRUCTOFURANOSIDASE FROM XANTHOPHYLLOMYCES DENDRORHOUS

M. Gimeno-Pérez[1], Z. Merdzo[1], C. Martín de las Hijas[1], L. Lutter[1], N. Míguez[2], M. Ramírez-Escudero[3], F.J. Plou[2], J. Sanz-Aparicio[3], M. Fernández-Lobato[1]
[1]Centro de Biología Molecular Severo Ochoa, Departamento de Biología Molecular, Madrid, Spain
[2]Instituto de Catálisis y Petroleoquímica, Grupo de Biocatálisis Aplicada, Madrid, Spain
[3]Instituto de Química Física Rocasolano, Departamento de Cristalografía y Biología Estructural, Madrid, Spain

Backgrounds

Promoting health through functional food consumption is a current target in food industry that demands the development of new products including bioactive compounds as prebiotics. In this context, some β-fructofuranosidases are biotechnologically interesting enzymes able to transfer fructose moieties to sucrose generating fructooligosaccharides (FOS). Among them, *Xanthophyllomyces dendrorhous* β-fructofuranosidase (Xd-INV) has the ability to transfer a fructose unit to D-glucose of sucrose through a β(2-6) linkage, forming the FOS neokestose and neonystose as main products. Structural analysis of this enzyme reveals an active high glycosylated dimer, of which monomers show the typical bimodular arrangement from the GH32 family with a long C-terminal extension and a flexible loop (Glu334 – His343) surrounding the catalytic pocket that accommodates substrates together with Trp105.

Objectives

Analysis of the role of relevant residues in the flexible loop and Trp105 that could modulate transfructosylating specificity of the enzyme.

Methods

Site-directed mutagenesis of Xd-INV (Trp105, Glu334, Gln341, Asn342, His343 residues) and heterologous expression of the mutant proteins in *Pichia pastoris* was developed. Kinetic behavior of Xd-INV variants were assessed using HPLC and the involved structural determinants enlightened.

Conclusions

In this study, we have obtained and analyzed diverse mutants of Xd-INV involved in the enzyme specificity and assigned a functional role to the peculiar flexible loop Glu334-His343 and Trp105 residue of this protein.

FEMS7-0534
Biotechnology / Synthetic Biology / Systems Biology - Part II

ANTIMICROBIAL POTENTIAL AND MECHANISM OF ACTION OF THYMOQUINONE AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

S. Goel[1], *P. Mishra*[1]
[1]*Indian Institute of Technology, Biochemical Engineering and Biotechnology, NEW DELHI, India*

Backgrounds

Antimicrobials of plant origin have been proved to be of paramount importance in combating the antibiotic resistance developed in pathogenic bacteria. One such phytochemical is Thymoquinone, extracted from *Nigella sativa*, which has shown potential antibacterial activity.

Objectives

To study Antibacterial potency and Anti-biofilm activity of thymoquinone and its underlying mechanism of action.

Methods

The antibacterial activity of Thymoquinone was quantified by calculating its MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration), Bactericidal kinetics (time required to kill bacteria) and Post-antibiotic effect (post treatment efficiency of inhibiting bacterial growth). Cytotoxicity of thymoquinone on HaCaT (keratinocytes) cell line was also studied. Also, the bactericidal activity of thymoquinone was visually studied by SEM and LIVE/DEAD fluorescent imaging.

Conclusions

Thymoquinone was found to be a very promising bactericidal and biofilm inhibiting agent against gram negative *(E. coli* and *P. aeruginosa*) and gram positive bacteria (*B. subtilis* and *S. aureus*). MIC and MBC values of thymoquinone were found to be very less and comparable to that of kanamycin and chloramphenicol.

SEM imaging showed cell wall surface irregularities and cell lysis with cellular content release. LIVE/DEAD imaging using acridine orange (AO) and ethidium dibromide (EtBr) confirmed the bactericidal activity as treated bacteria showed selective uptake of EtBr over AO.

We find that Thymoquinone acts by ROS induction in cells, as shown by H_2DCF-DA assay.

In addition, Thymoquinone, at MIC, was found to be nontoxic to human keratinocytes (except for MIC value of *E.coli*) demonstrating its efficacy as a promising antibacterial agent.

FEMS7-1842
Biotechnology / Synthetic Biology / Systems Biology - Part II

DECIPHERING LIGNIN METABOLISM IN PSEUDOMONAS PUTIDA

H. Gomez-Alvarez[1], *Z. Martin-Moldes*[1], *J. Nogales*[1], *E. Diaz*[1]
[1]*Centro de Investigaciones Biologicas-CSIC, Department of Environmental Biology, Madrid, Spain*

Backgrounds

Lignin is discarded in paper manufacturing and biofuel production processes in spite of its peerless potential as a renewable source of aromatics for the biochemical industry. The undeniable bottleneck for its utilization relies on the development of an efficient depolymerising strategy that translates lignin molecular complexity into monoaromatics. A few bacteria are so far regarded as potential *whole-package chassis* for lignin valorisation given their ligninolytic capacities, metabolic versatility and ease to be engineered[1,2]. *Pseudomonas putida* KT2440 is one of them, and its potential as a lignin-based biochemical producer has already been described[3]. Nonetheless, an indisputable lack of knowledge regarding lignin depolymerisation by bacteria hampers proper lignin exploitation and stresses the need for further studies on bacterial lignin metabolism.

1 http://dx.doi.org/10.1016/j.cbpa.2015.06.009

2 http://dx.doi.org/10.1016/j.copbio.2016.02.030

3 http://dx.doi.org/10.1073/pnas.1410657111

Objectives

To achieve a systems biology view of the lignin metabolism in *Pseudomonas putida* KT2440 as basis for engineering future lignin valorisation processes.

Methods

A systems biology workflow including transcriptomics, genetic engineering, and biochemical analytical methods combined with metabolic reconstruction is applied to develop a lignin-specific metabolic model of *P. putida* KT2440. The metabolic model is then employed to better identify and overcome limitations in lignin utilization.

Conclusions

The lignin-specific metabolism of *Pseudomonas putida* KT2440 will be described, with special emphasis in those pathways likely involved in high-molecular-weight lignin depolymerisation. Model-driven metabolic engineering strategies for optimal lignin metabolism will also be presented. In overall, this work illustrates the metabolic capacities of *Pseudomonas putida* KT2440 towards lignin utilization and paves the way for future valorisation processes.

FEMS7-1945
Biotechnology / Synthetic Biology / Systems Biology - Part II

INNOVATION IN BACTERIOPHAGE-BASED BIOCONTROL OF THE PLANT PATHOGEN RALSTONIA SOLANACEARUM THROUGH IRRIGATION WATER

E. G Biosca[1], *B. Álvarez*[1,2], *M.M. López*[2]
[1]*Universitat de Valencia, Microbiología y Ecología, Burjassot, Spain*
[2]*Instituto Valenciano de Investigaciones Agrarias IVIA, Bacteriología, Moncada, Spain*

Backgrounds

Ralstonia solanacearum is a soil- and water-borne plant pathogen responsible for bacterial wilt, one of the most devastating bacterial diseases of solanaceous crops. This pathogen has a quarantine status in the European Union (EU) where it has been frequently detected in waterways, and associated with several water-borne outbreaks. This poses a problem for growers because the use of *R. solanacearum* contaminated water for irrigation is prohibited in the EU, where water scarcity is increasing, particularly in Mediterranean countries.

Objectives

The objective was to develop an innovative biocontrol procedure based on the lytic action of *R. solanacearum* specific bacteriophages for bacterial wilt disease management through irrigation water.

Methods

Lytic bacteriophages of *R. solanacearum* were isolated from environmental water and selected according to their specificity, stability and lytic activity under different environmental conditions. Morphology, and molecular and genomic characteristics were also determined. Biocontrol ability was assessed in irrigation water and host plants watered with the pathogen alone or with different combinations of the bacteriophages, also after their production at pilot scale.

Conclusions

The innovative technology developed, which is patent pending (priority number P201530730), reduces bacterial wilt incidence in all cases, with disease absence in most of them. It offers a natural, efficient and easily applicable strategy for bacterial wilt prevention and/or control, with less legal restrictions and environmental impact than chemical treatments. It can also be incorporated into integrated management programs against bacterial wilt. (CPI_14_244_Valoritza i transfereix_VLC/CAMPUS_UVEG & IVIA).

FEMS7-2282
Biotechnology / Synthetic Biology / Systems Biology - Part II

ANTIMICROBIAL ACTIVITIES AND ANALYSIS OF THE SECONDARY METABOLITES PRODUCED BY TWO AMYCOLATOPSIS STRAINS ISOLATED FROM LICHENS.

I. Gonzalez Martinez[1], F. Román[1], M. Estévez[1], F. Muñoz[2], C. Justicia[2], M. de la Cruz[3], P. Sánchez[3], L. Rodríguez[3], B. Cautain[3], J. Martín[2], J. Tormo[2], O. Genilloud[1]
[1]Fundacion MEDINA, Microbiology, Granada, Spain
[2]Fundacion MEDINA, Chemistry, Granada, Spain
[3]Fundacion MEDINA, Screening, Granada, Spain

Backgrounds

Microbes are the leading producers of useful natural products. For decades, microbial natural products have been one of the major sources of novel drugs for pharmaceutical companies, and today there still are novel molecules waiting to be discovered from these natural sources, especially from actinomycetes.

Objectives

Analysis of the secondary metabolites with antimicrobial activity produced by two *Amycolatopsis* strains. Fermentation of the strains in two different formats and in 8 media. Test crude extracts against a panel of Gram-positive and Gram-negative bacteria and two fungi. Bioassay guided fractionation by HPLC of the active extracts. Identification of the active compounds by LCMS analysis.

Methods

We fermented two *Amycolatopsis* strains in two fermentation formats, and we studied their antimicrobial, antibacterial and antifungal activities and cytotoxicity. The strains were fermented in eight media from which we selected the medium FRM for scale-up since the strains produced more secondary metabolites with activity against Gram-negative bacteria. The scale-up extract was fractionated by HPLC, and tested to confirm the original activity. The active fractions were analyzed by LC-MS.

Conclusions

One of the strains produced a family of novel antibiotics with activity against Gram-negative bacteria and structurally related to macrolides, which did not have any coincidence to any entry in the Dictionary of Natural Products,. Another novel chlorinated compound was detected by LC-MS, which could have a role in the activity on some of the microbes of the panel.

FEMS7-1637
Biotechnology / Synthetic Biology / Systems Biology - Part II

ENHANCED PRODUCTION OF CERULENIN BY A NEW FUNGAL SPECIES OF PHOMA FOLLOWING IMPROVED FERMENTATION CONDITIONS

V. Gonzalez-Menendez[1], *G. Crespo*[2], *C. Toro*[1], *F. Muñoz*[2], *J. Martin*[2], *F. Reyes*[2], *O. Genilloud*[1], *J.R. Tormo*[2]
[1]*Fundacion MEDINA, Microbiology, Granada, Spain*
[2]*Fundacion MEDINA, Chemistry, Granada, Spain*

Backgrounds

As result of an antimicrobial Natural Products Screening campaign an endophytic fungal strain of *Phoma* sp. isolated from the endemic plant *Arthrocnemun macrostachyum* from the saltmarsh of Cabo de Gata (Almeria) was shown to produce cerulenin as determined by LC/MS dereplication. Cerulenin is a commercial inhibitor of the fatty acid and polyketide synthases with known antimicrobial properties. This antibiotic had only been reported previously as produced by the rice pathogen *Sarocladium oryzae* from which all efforts in trying to improve production and increase cerulenin fermentation titers in this strain had limited success.

Objectives

Evaluate and improve the production of cerulenin in a new producing strain following an OSMAC approach to enhance the metabolite production.

Methods

Test the use of adsorptive polymeric resins and small-molecule epigenetic elicitors in an OSMAC approach to enhance cerulenin production by the new *Phoma* sp. strain.

Conclusions

The use of epigenetic modifiers did not increase titers of the target compound, but induced the production of three different dendrolides in the presence of 5-azacitidine and valproic acid. On the contrary, the addition of XAD-16 resin did result in a 5-fold increase in the production of cerulenin compared to the best previous production conditions described (the original producer strain in a zeolite based fermentation with an ion trapped agent). The production of cerulenin by this new producer strain, offers an alternative solution for the production of cerulenin with better yields.

FEMS7-2879
Biotechnology / Synthetic Biology / Systems Biology - Part II

A NOVEL COMPUTATIONAL METHOD TO IMPROVE BIOTECHNOLOGICAL PROCESSES BASED ON MICROBIAL COMMUNITIES

A. Sorokin[4], A. Orakov[1,5], N. Sakenova[1,5], I. Goryanin[1,2,3]
[1]OIST, Biological Systems Unit, Onna, Japan
[2]University of Edinburgh, School of Informatics, Edinburgh, United Kingdom
[3]Tianjin Institue of Industrial Biotechnology, Biodesign centre, Tianjin, China
[4]Institute of Cell Biophysics RAS, Mechanism of Cell Genome Functioning Laboratory, Pushchino, Russia
[5]School of Science and Technology, Nazarbayev University, Astana, Kazakhstan

Background
There is a great potential for the use of bacterial communities for bioremediation and waste treatment. Systems metabolic engineering for microbial community redesign is a promising approach. The successful development of synthetic microbial communities is essential to add value to the main challenges for sustainable development. Some of these challenges could be addressed by advances in computational tools and modelling. However, current tools only provide separate taxonomic and functional analyses.

Objectives
A number of experimental and in silico tools have been produced, but they need to be further improved. The challenge is to create effective integrated functional, taxonomic and metabolic analyses.

Methods
Our post-annotation analysis and visualization tool uses data integration algorithm to merge taxonomic and functional data annotated at read level. The resulting 3D dataset with axes of Functions, Taxonomy and Metagenome samples is visualized via three heatmaps of each axis versus two others (F&T, F&M, T&M). Additionally, KEGG pathway enrichment sorting/heatmap and its map visualization are implemented.

Conclusions
Advantages of the tool are: 1) Integrated functional and taxonomic analysis; 2) Comparative analysis of KEGG pathway enrichments; 3) KEGG pathway maps; 4) Both DNA and RNA analyses. We have applied our method to analyze metagenomic data from Microbial Fuel Cell communities which are used for wastewater treatment. We have identified set of genes which should be knocked down or overexpressed to improve treatment performance. We are going to test our predictions to improve real waste treatment from awamori (sake) plants and swine farms in Okinawa.

FEMS7-1204
Biotechnology / Synthetic Biology / Systems Biology - Part II

IDENTIFICATION OF WILD YEASTS IN ECUADORIAN CHICHAS

N.J. Grijalva Vallejos[1], E. Matallana[1], A. Aranda[2]
[1]Universitat de Valencia, Biochemistry and Molecular Biology, Valencia- Burjassot, Spain
[2]Instituto de Agroquímica y Tecnología de Alimentos CSIC,
Departamento de Biotecnología de Alimentos, Valencia, Spain

Backgrounds

Ecuadorian *chichas* are a variety of homemade beverages with a low alcoholic content, derived from the spontaneous fermentation of cereals (mainly maize) and/or fruits. These substrates are important habitats for bacteria and wild yeast populations that have not been fully characterized. Some of them may be related with specific organoleptic characteristics of the final product.

Objectives

The aim of this study was to identify different species of yeast with fermentative potential, present in the natural microbiota of four Ecuadorian *chichas* of rice, oats, grape and *yamor* (traditional indigenous beverage which is brewed using seven varieties of corn. It is known as the drink of Wisdom).

Methods

Samples of the four types of Ecuadorian *chichas,* prepared under controlled conditions, were taken at three time-points of fermentation: initial (day 3), tumultuous (day 7) and final (day 14). A total of 254 yeast isolates were identified by conventional microbiological analysis and by polymerase chain reaction – restriction fragment length polymorfism (PCR – RFLP) of ITS1-5.8S rDNA – ITS2; results were confirmed by sequencing. Interdelta analysis for *Saccharomyces cerevisiae* strain characterization was also performed.

Conclusions

Twelve yeast genera were isolated from Ecuadorian *chichas* samples. The most representative yeast species were: *Hanseniaspora guillermondii*, *Hanseniaspora opuntiae, Saccharomyces cerevisiae, Torulaspora delbruecky, Pichia kluyveri, Candida sp.* and *Rhodotorula mucilaginosa. Hanseniaspora* species were dominant at the beginning of fermentation in all the analyzed beverages. *Saccharomyces cerevisiae* isolates were abundant in rice *chicha* and *Torulaspora delbruecky* isolates dominates in grape *chicha*. In further investigations we will study the potential industrial applications of these new yeast strains.

FEMS7-1427
Biotechnology / Synthetic Biology / Systems Biology - Part II

CRISPR-CAS9 TARGETING OF THE E. COLI CHROMOSOME REVEALS LARGE-SCALE SENSITIVITY PATTERNS

B. Gutierrez[1], *L. Cui*[1], *A. Bernheim*[1], *D. Bikard*[1]
[1]*Institut Pasteur, Microbiology, Paris, France*

Backgrounds

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) loci and their Cas (CRISPR associated) proteins have been shown to provide an adaptive immunity in bacteria. They are able to capture DNA fragments of plasmids, phages and other invading elements and use this information to destroy homologous genetic elements. When programmed to target the bacterial chromosome, CRISPR-Cas9 was shown to kill the cell, and this property could be optimized to engineer sequence-specific antimicrobials. Nevertheless, the efficiency with which Cas9 can find and cleave its target is not always the same but likely depends on the target sequence and position along the genome.

Objectives

The goal of this study is to elucidate which are the genetic requirements for efficient CRISPR-Cas9 targeting in the bacterial chromosome.

Methods

We have performed a high-throughput screening with a CRISPR library that we constructed including 10^5 different guide RNAs targeting the chromosome of *Escherichia coli*. This library was introduced in *E. coli* and the expression of Cas9 was induced. In order to determine the efficiency with which each guide RNA can kill *E. coli,* next generation sequencing of the library was performed before and after induction.

Conclusions

According to our results, there are efficient guide RNAs, which are rapidly depleted from the library, while weak guide RNAs, are still present several hours after Cas9 induction. This experiment revealed a large-scale pattern in which large regions of the chromosome are more susceptible to the Cas9-mediated killing.

FEMS7-2675
Biotechnology / Synthetic Biology / Systems Biology - Part II

E. COLI CHASSIS VARIANTS WITH ENHANCED TRANSLATIONAL CAPACITY: MANIPULATION OF THE GENOMIC COPY NUMBER OF RRNA AND TRNA GENES

Z. Gyorfy[1], Z. Lipinszki[1], V. Vernyik[1], G. Draskovits[1], K. Urmos[1], T. Sari[1], F. Blattner[2], G. Posfai[1]
[1]Biological Research Center, Institute of Biochemistry, Szeged, Hungary
[2]Scarab Genomics, LLC, Madison, USA

Backgrounds

Translation of recombinant proteins in a prokaryotic host is often limited by the capacity of the translational apparatus of the host. By adjusting the abundance of key components (rRNA operons, tRNA genes) of the host, we sought to enhance the expression of recombinant proteins in *E. coli*.

Objectives

First, we investigated the effect of changing the wt number of rRNA operons. Testing for GFP production, we found that increased rRNA operon copy number resulted in increased amount of GFP per cell. On the population level, however, lower copy numbers were beneficial. We showed that while higher GFP production per cell in high copy number variants was due to increased cell size, slower growth (due to fewer rRNA operon copies) allowed for higher GFP/total protein ratio. Next, we doubled the copies of six rare tRNA genes for expression of recombinant proteins with suboptimal codon composition. The novelty of the approach was to insert the tRNA genes into rRNA operons. This arrangement ensures that extra tRNA is supplied on demand (depending on growth rate), minimizing interference with normal cell physiology. Insertion of the tRNA genes resulted in increased expression of GFP variants carrying runs of rare codons, and demonstrated the advantage of the approach over traditional, plasmid-based tRNA abundance adjustments.

Methods

Genomes with various numbers of rRNA operons and tRNA genes were constructed by homologous recombination-based procedures.

Conclusions

E. coli variants with adjusted numbers of rRNA operons and tRNA genes may be useful alternatives for enhanced recombinant protein production.

FEMS7-2144
Biotechnology / Synthetic Biology / Systems Biology - Part II

DEVELOPMENT OF SELF-HEALING TECHNIQUE FOR CONCRETE CRACKING BY USING GLYCOCALYX SLIME-PRODUCING AND AUTOTROPHIC BACTERIA

E.S. Ha[1], K.H. Yang[2], S.S. Lee[1]
[1]Kyonggi University, Life Science, Suwon, Republic of Korea
[2]Kyonggi University, Plant-Architectural Engineering, Suwon, Republic of Korea

Backgrounds

Development of self-healing concrete is required for recover concrete-cracking because the technique can solve the problems in economic aspect.

Objectives

This study is to develop a self-healing concrete technique for cracking during the long term by using, which can be tolerance to strong alkali environment. Moreover, we searched the optimal adsorption condition.

Methods

Glycocalyx slime producing and autotrophic bacteria were identified as *Rhodobacter capsulatus*, *Rhodopseudomonas palustris, Rhodobacter blasticus, Rhodospirillum rubrum, Rhodobacter sphaeroids* with 1ml of each strain and 9ml of each culture. Glycocalyx slime producing was confirmed by incubation of strains with five kinds of carbon source (succinate, fructose, glucose, lactate, malate) at 25℃ under light.

The bacterial growth was confirmed in OD_{600} and glycocalyx slime was extracted and quantified in weight. The amount of slime produced in malic acid - treated culture medium was the highest in Rhodobacter blasticus strains (1.47 ± 0.08 g / L) except for Rhodospirillum rubrum. It was recommended as an optimal strain by showing a film formation efficiency considerably higher than the first target (0.5 g / L). Meanwhile, bacteria adsorbed four materials such as powder-formed high absorbent resin, bead formed high absorbent resin, expanded vermiculite and phyllite. Form SEM analysis, we confirmed cation exchange capacity and absorption of bacteria on the materials. Bacterial growth adsorbed on expanded vermiculite was optimal in 120 ~ 150 meq / 100 g and pH in range of 5.5 ~ 7.5. The optimal material is the magnesia phosphate potassium in determination of various phosphate sources with magnesia. The water to binder for material is 40%. Magnesia molar phosphate potassium complex is 3.4. As Molar magnesia phosphate has decreased, TruByte-K increased and the pore structure tended to become dense.

Conclusions

The technique could be applied to develop self-healing concrete in the civil engineering or plant-architecture engineering field.

FEMS7-2993
Biotechnology / Synthetic Biology / Systems Biology - Part II

SYSTEMS ANALYSIS AND GENOME SCALE METABOLIC NETWORK RECONSTRUCTION IN PSEUDOMONAS VERONII 1YDBTEX2 FOR INTEGRATION OF GENOME-WIDE EXPRESSION DATA AND PHYSIOLOGY INTERPRETATION

N. Hadadi[1], A. Chiappino Pepe[1], V. Pandey[1], M. Morales[2], M. Dubey[2], J.R. van der Meer[2], V. Hatzimanikatis[1]
[1]Ecole Polytechnique Fédérale de Lausane, Chemistry and Chemical Engineering, Lausanne, Switzerland
[2]University of Lausanne, Department of Fundamental Microbiology, Lausanne, Switzerland

Backgrounds

Pseudomonas veronii is a motile bacterium capable of degrading a variety of aromatic hydrocarbons such as benzene, toluene, ethylbenzene and *m*- and *p*-xylene (BTEX). Additionally, the strain *P. veronii* 1YdBTEX2 can survive on a minimal growth medium that contains an aromatic compound as a single carbon source. The growth of the bacterium in contaminated environments makes it a promising natural tool for bioremediation of contaminated soils.

Objectives

In this study, we investigated the metabolic flexibility and versatility of *P. veronii* 1YdBTEX2 at system level through the reconstruction and analysis of its genome-scale metabolic model (GEM). GEMs serve as platforms to integrate context specific data (i.e., omics data), to predict strain-specific phenotypes, and ultimately allow for better understanding of genotype-phenotype relationships.

Methods

We used the RAVEN Toolbox to reconstruct *itPvr*, the first genome-scale metabolic model of *P. veronii*.

Conclusions

itPvr accounts for 1243 genes, 1738 reactions, and 1795 metabolites. We performed extensive and manually curated gap-filling and quality control to reconcile experimental data such as growth measurements on different media and genome-wide expression data obtained under various exposures.

We further performed bioenergetics analysis to investigate the effect of thermodynamics constraints on the feasibility of reactions and on the metabolic capabilities.

We demonstrate how *itPvr* can serve as a platform for predicting cellular responses to environmental or genetic perturbations. *itPvr* provides a holistic overview of the metabolic needs and capacities of *P. veronii* and can guide experimental strategies to enhance the biodegradation capabilities of *P. veronii* to be ultimately used for bioremediation of contaminated soils.

FEMS7-2019
Biotechnology / Synthetic Biology / Systems Biology - Part II

SUNLIGHT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES BY YARROWIA LIPOLYTICA NBRC 1658 AND SACCHAROMYCES CEREVISIAE AND DETERMINATION OF ANTIMICROBIAL ACTIVITY

H. hammamchi[1], *G. Koşarsoy Ağçeli*[1], *N. Cihangir*[1]
[1]*Hacettepe, Biology, Ankara, Turkey*

Backgrounds

Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances. Silver nanoparticles have potential to be using in several biological applications.

Objectives

Silver nanoparticles used in this present study were synthesized by standard published procedures with little modifications. Therefore, we focused on the biological synthesis silver nanoparticles from *Yarrowia lipolytica* NBRC 1658 and *Saccharomyces cerevisiae*.

Methods

Yeast cultures were grown in yeast extract broth and centrifuged at 72,000 × g for 20 minutes. Supernatant was mixed with 1 mM $AgNO_3$ solution for the synthesis of AgNPs and incubated at room temperature sunlight conditions. The different parameters were optimized for the synthesis of AgNPs including concentration of silver nitrate, concentration of yeast extract and silver nitrate, time, temperature and pH which had been identified as factors which affect the productivity of AgNPs. Silver nanoparticles were analysed using UV–Vis spectrophotometer and Fourier Transform Infrared Spectroscopy (FTIR), SEM, XRD. There antibacterial activites checked by disc diffusion method. The antimicrobial index compared with other antibiotics.

Conclusions

The obtained results clearly suggest that microorganisms have potential to synthesis of eco-freindly Silver nanoparticles and using in several application.

FEMS7-2101
Biotechnology / Synthetic Biology / Systems Biology - Part II

EXTRACELLULAR SYNTHESIS OF SILVER NANOPARTICLES BY MONASCUS SP. AND ITS BIOMEDICAL APPLICATIONS

H. hamideh[1], G. Koşarsoy Ağçeli[1], N. Cihangir[1]
[1]hacettepe, biology, ankara, Turkey

Backgrounds

Nanotechnology is rapidly growing field and deals with synthesis and control the materials with nano size ranges from 1 to 100 nm. Microorganisms have potential to be using in several biological applications, such as enzyme production, nanopartical synthesis.

Objectives

The main object of the work was biological synthesis silver nanoparticles by a fungus, Monascus sp.

Methods

In this work fungal cultures were grown in malt extract broth, and incubated at 30^{o}C. The different parameters were optimized for the synthesis of SNPs including concentration of silver nitrate, concentration ratio of fungal extract and silver nitrate, time, temperature and pH which had been identified as factors which affect the productivity of SNPs. The biomass was harvested by using Whatman filter paper No. 1 and washed twice with sterile double distilled water to remove any medium component from the biomass. The filtrate was challenged with 1mM of $AgNO_3$ and incubated at 25^{o}C under dark and light room condition. The color of the solution slowly turned black-dark yellow indicating the reduction of silver ions and the formation of the AgNPs. The AgNPs were characterized using UV–Visible spectroscopy, SEM, FTIR, XRD, AFM. The activity of biologically synthesised silver nanoparticles (AgNPs) was checked by disc diffusion method for using antibacterial ageint.

Conclusions

In conclusion, we have reported biological synthesis of AgNPs using Monascus sp. as a green, reusable, nontoxicand inexpensive heterogeneous catalyst. Thesynthesized AgNPs were characterized by using UV–vis,XRD, FTIR, AFM and SEM and the bacterial biosynthesis of the titaniumdioxide provides a fast, purest form of producing nanoparticles.

FEMS7-0358
Biotechnology / Synthetic Biology / Systems Biology - Part II

X-RAY STRUCTURE OF LINALOOL DEHYDRATASE/ISOMERASE REVEALS ENZYMATIC ALKENE SYNTHESIS- AN ENZYME FOR THE BIOTECHNOLOGICAL PRODUCTION OF BUTADIENE?

J. Harder[1], *R. Marmulla*[1], *S. Weidenweber*[2], *U. Ermler*[2]
[1]*Max-Planck-Institute for Marine Microbiology, Microbiology, Bremen, Germany*
[2]*Max-Planck-Institute of Biophysics, Molecular Membrane Biology, Frankfurt, Germany*

Backgrounds

Linalool dehydratase/isomerase (Ldi), an enzyme of terpene degradation in *Castellaniella defragrans*, isomerizes the primary monoterpene alcohol geraniol into the tertiary alcohol (S)-linalool and dehydrates (S)-linalool to the alkene b-myrcene. Future biotechnological applications of Ldi are in particular the industrial butadiene and isoprene production from renewable sources. Over twenty patents have reported the potential use of the enzyme for such a biotechnological application which may lead to nylon and other polymers as well as to octane and other biofuels.

Objectives

Here we report on the crystal structures of Ldi with and without terpene substrates, revealing a cofactor-free homopentameric enzyme. The substrates were embedded inside a hydrophobic channel between two monomers of the (alpha,alpha)6 barrel fold class and flanked by three clusters of polar residues involved in acid-base catalysis.

Methods

The enzyme was purified after expression in *Escherichia coli* and crystalized for structure determination. Amino acid mutants were constructed and tested for activity.

Conclusions

The presented structural data of Ldi revealed the terpene binding site between two monomers inside a hydrophobic channel, unprecedentedly for (alpha,alpha)6 barrel proteins, and three catalytic clusters involved in catalysis. This knowledge provides a rational basis for expanding the substrate specificity of the enzyme and for increasing its turnover rate. From the new platform more targeted biotechnological avenues can be explored.

FEMS7-2025
Biotechnology / Synthetic Biology / Systems Biology - Part II

FUNGALBRAID: A GOLDENBRAID-BASED MODULAR CLONING PLATFORM FOR THE ASSEMBLY AND EXCHANGE OF DNA ELEMENTS TAILORED TO FUNGAL BIOTECHNOLOGY

M. Hernanz-Koers[1], M. Gandía[1], G. Sandra[1], M. Paloma[1], L. Yenush[2], D. Orzáez[3], J.F. Marcos[1]
[1]Instituto de Agroquímica y Tecnología de los Alimentos IATA-CSIC, Department of Food Biotechnology, Paterna, Spain
[2]Instituto de Biología Molecular y Celular de Plantas IBMCP, Mecanismos de la Respuesta al Estrés en Plantas, Valencia, Spain
[3]Instituto de Biología Molecular y Celular de Plantas IBMCP, Biotecnología y Mejora Vegetal de Especies Cultivadas, Valencia, Spain

Backgrounds

Challenges ahead in the study and exploitation of filamentous fungi are the optimization of DNA cloning and fungal genetic transformation, the exchange of standardized genetic elements, and the availability of synthetic biology tools.

Objectives

In this context, we propose the adaptation of the plant bio-design platform GoldenBraid 3.0 (GB) to fungi through: (i) the expansion of the GB toolbox with the domestication of fungal-specific genetic elements; (ii) the design of specific GB structures; and (iii) the *Agrobacterium*-mediated genetic transformation (ATMT) and gene disruption of the plant pathogen *Penicillium digitatum* as proof of concept.

Methods

Genetic elements domesticated into the GB entry vector pUPD2 were: the promoters P*trpC* and P*gpdA* from *A. nidulans*, and P*paf* from *P. chrysogenum*; the positive selection marker *nat1* for Nou^R; the negative marker HSV*tk* for $F2dU^s$; and the terminators T*trpC* from *A. nidulans*, T*tub* from *N. crassa* and P*paf* from *P. chrysogenum*. Other GB elements can be directly used in fungi, such as the markers *hph* for Hyg^R or *nptII* for $G418^R$, fluorescent protein reporters or epitope tags. Modular assembly of elements generates an increasing number of diverse transcriptional units in the pDGBα3/pDGBΩ3 destination vectors. The GB grammar was adapted to design specific GB structures for gene disruption through homologous recombination and dual selection. Furthermore, universal primers were designed for the analysis of transformants. ATMT of *P. digitatum* resulted in positive transformants.

Conclusions

The GoldenBraid technology has been successfully adapted for the ATMT of filamentous fungi. We propose the name of FungalBraid (FB) for this new branch of GB.

FEMS7-3082
Biotechnology / Synthetic Biology / Systems Biology - Part II

STRUCTURAL DESIGN OF A CHIMERIC MULTIVALENT NEISSERIA MENINGITIDIS VACCINE

S. Hollingshead[1], I. Jongerius[2], S. Johnson[1], R. Exley[1], S. Lea[1], C. Tang[1]
[1]University of Oxford, Sir William Dunn School of Pathology, Oxford, United Kingdom
[2]UMC Utrecht, Bacterial Infections and Immunity, Utecht, The Netherlands

Backgrounds

Serogroup B *Neisseria meningitidis* is the predominant cause of meningococcal disease in the Western hemisphere. A polysaccharide vaccine for this serogroup is not feasible due to its poor immunogenicity. Therefore alternative approaches are required to generate a serogroup B meningococcal vaccine.

Objectives

We used structure based design to construct a chimeric vaccine composed of the meningococcal antigens fHbp and PorA.

Methods

fHbp was used as a molecular scaffold to display the immunogenic VR2 loop of PorA. We successfully generated several fHbp-PorA chimeras that retained both the fHbp and PorA antigenic epitopes and induced an immune response against both antigens. Our biochemical analyses and fHbp-PorA structures, demonstrate the fHbp-PorA chimeras are thermally stable, elicit bactericidal antibodies directed against fHbp and PorA, and the PorA epitope folds in a conformation recognised by a bactericidal antibody.

Conclusions

The work provides the proof in principle that fHbp can be used as a molecular scaffold upon which surface exposed regions of any immunogenic membrane protein, such as PorA, can be displayed. Similar approaches could be adopted for other pathogens.

FEMS7-0383
Biotechnology / Synthetic Biology / Systems Biology - Part II

RAISING BUTANOL PRODUCTION FROM AROMATIC COMPOUNDS WITH METABOLIC MODIFICATION IN RHODOCOCCUS JOSTII RHA1

B.S. Hsieh[1], H.M. Yang[1], G.H. Lin[2]
[1]Tzu Chi University, Master Program in Microbiology and Immunology, Hualien, Taiwan R.O.C.
[2]Tzu Chi University, Department of Microbiology- School of Medicine, Hualien, Taiwan R.O.C.

Backgrounds

The problem of aromatic compounds pollution and shortage of petroleum arise with the industrial development. *Rhodococcus jostii* RHA1 is able to degrade aromatic compounds due to lots of oxygenases in it. No existing microorganism has been reported to remove the toxic substances and synthesize the biofuel simultaneously.

Objectives

The aim of this study is to produce the butanol from the consumption of the aromatic compounds and elevate the butanol production with metabolic engineering in *R. jostii* RHA1.

Methods

R. jostii RHA1BuOH was construct by overexpression of heterologous KIVD(Alpha-ketoisovalerate decarboxylase from *Lactobacillus lactis*) and ADH2(alcohol dehydrogenase2 from *Saccharomyces cerevisiae*) in *R. jostii* RHA1,.and *R. jostii* RHA1$P_{ro04166}$TFBS BuOH was construct by replacing the promoter with the native promoter $P_{ro04166\ TF\ BS}$ in *R. jostii* RHA1, The butanol production from both strains is optimization by metabolic modification in keto-acid pathways with markerless mutagenesis. The produced alcohol compounds were quantified by a gas chromatograph.

Conclusions

The results suggested that the butanol was produced by using environmental contaminants and lignocellulose with *R. jostii* RHA1$P_{ro04166}$TFBSBuOH. The modified strain, *R. jostii* RHA1BuOH*1,* was construct, but the quantity of alcohols compounds have not been determined by a gas chromatograph.

FEMS7-0503
Biotechnology / Synthetic Biology / Systems Biology - Part II

PSEUDOMONAS PUTIDA WSP COMPLEX AND PSEUDOMONAS AERUGINOSA PELA PROMOTER AS THE COMPONENTS OF A CONTACT DEPENDENT SURFACE SWITCH

A. Hueso-Gil[1], V. de Lorenzo[1]
[1]Centro Nacional de Biotecnología, Systems Biology, Madrid, Spain

Backgrounds

As many other microorganisms, *Pseudomonas putida* senses surfaces and changes its lifestyle from planktonic to biofilms mainly through a whole of physiological changes orchestrated by the endogenous secondary messenger c-di-GMP.

Objectives

This state of affairs opens the possibility to construct a surface-responsive device where contact sensing by the bacteria is connected to a desired output through the effect of c-di-GMP.

Methods

We have characterized a surface protein complex, Wsp, homologous to the counterpart in the *P. putida* relative, *P. aeruginosa* (Hickman *et al*., 2005) in terms of colony morphology, c-di-GMP quantification, biofilm formation.... We have also developed a c-di-GMP reporter that can sense variations in c-di-GMP using the promoter of the *pelA* gene of *P. aeruginosa* (Baraquet *et al*., 2012), the first gene of the *pel* operon. This promoter has been engineered upstream of *lacZ*

Conclusions

Similarly to the species where it was first found, the Wsp system of *P. putida* is composed of several structural components and two main proteins: WspR, which encodes the domains GGDEF and EAL (responsible of cycling or esterifying c-di-GMP, respectively), and WspF, the protein which regulates WspR activity due to its methylesterase activity (Huangyutitham *et al*., 2013). Regarding to the promoter, In *P. putida,* P_{pelA} is regulated mainly through repression of the native FleQ regulator protein, while FleN has only a minor role. This is in contrast to the functioning of the pair FleQ-FleN that regulates expression of *pelA* gene in *P. aeruginosa* by both repression and activation.

FEMS7-1426
Biotechnology / Synthetic Biology / Systems Biology - Part II

MICROBIAL FUEL CELL OPERATING WITH THIOSULFATE AND AN ACIDOPHILIC MIXED CULTURE

J. Hurtado[1], S. Jibaja[1], J. Medina[2], J. Begazo[1], A. Berastain[1]
[1]Universidad Peruana Cayetano Heredia, Ciencias celulares y moleculares, Lima, Peru
[2]Universidad Peruana Cayetano Heredia, Ciencias Exactas, Lima, Peru

Backgrounds

Microbial fuel cells (MFC´s) have been demonstrated using a variety of microorganisms including acidophilic microorganisms. Bacterial leaching often realease metlas and and inorganic sulfur compounds as tetrathionate and thiosulfate to the mining process and waste waters. The untreated acidic metal-rich water may harm the environment. It has been reported generation of electricity in microbial fuel cells using tetrathionate, but not using thiosulfate. Here, we investigate the use of thiosulfate in microbial fuel cells using an acidophilic mixed culture.

Objectives

Demonstrate the use of thiosulfate in microbial fuel cell using and acidophilic mixed culture

Methods

The fuel cell used in this study was a two-chamber camera with anode and cathode chambers separated by a Naflon 117 membrane. The electrodes were Graphite felt. The anolyte was thiosulfate at pH 3.6 and the catholyte was water. A mixed culture of anaerobic acidophilic bacteria growing in thiosulfate, developed from copper sulfides was used as inoculum. The maximum cell voltage obtained was 18 mV.

Conclusions

This study demonstrated that thiosulfate generate electricity using a acidophilic bacterial cell culture.

FEMS7-2739
Biotechnology / Synthetic Biology / Systems Biology - Part II

ISOLATION AND CHARACTERIZATION OF SULFUR-OXYDIZING BACTERIA (SOB)

A. Ibáñez[1], R. Cobos[1], A. Diez[1], S. González-García[1], J.M. Álvarez-Pérez[1], C. Barreiro[2], E. Garzón-Jimeno[1], J.J.R. Coque[1]
[1]Universidad de León, Instituto de Investigación de la Viña y el Vino, León, Spain
[2]INBIOTEC- Instituto de Biotecnología de León, Proteomics, León, Spain

Backgrounds

Phosphorous is the second most important key element as a mineral source in plant nutrition. Nevertheless, P is not easily available because it occurs mostly in insoluble forms, therefore large amounts of soluble forms of P are applied to soils as fertilizers. Furthermore, they are dependent on P, derived from phosphate rock, which is a finite resource, and based on its current rate of use, it has been estimated that the global worldwide known reserves of P rock may be depleted in approximately 100 years. The development of profitable eco-friendly technologies by using SOB (Sulphur-Oxidizing Bacteria), which oxidize sulphur to produce sulphuric acid creating an acidic leaching environment for phosphate solubilisation could contribute to the recovery of soluble phosphorous from phosphate rock.

Objectives

The isolation and characterization of SOB, and the analysis of their ability to obtain soluble phosphate from landfill schlams.

Methods

The SOB were isolated from different samples by using 4 different media: 9K-Fe, 9K-$Na_2S_2O_3$, *Acidithiobacillus thiooxidans* medium and *Leptospirillum* medium. Isolates were placed on *Thiobacillus* agar plates supplemented with bromocresol green as pH indicator and identified by amplification and sequencing of the 16S rDNA. The phosphate solubilized in a mini-real dump was simulated and measured with green malachite.

Conclusions

As a result of the experiments, the possibility of extracting the residual phosphorous of schlams in the long term by using SOB strains was determined. In the future, it will be advisable to find a sulphur mineral source in order to monetize the process.

FEMS7-2740
Biotechnology / Synthetic Biology / Systems Biology - Part II

DESIGN OF SCAR-TYPE MARKERS FOR DETECTION OF ACTINOBACTERIA WITH BIOCONTROL ACTIVITY AGAINST FUNGAL PATHOGENS BY QPCR

S. González-García[1], J.M. Álvarez-Pérez[1], A.M. Ibáñez Sánchez[1], R. Cobos[2], M.Á. Olego[1], A. Diez[2], E. Garzón-Jimeno[1], J.J.R. Coque[1]
[1]Universidad de León, Instituto de Investigación de la Viña y el Vino, León, Spain
[2]RGA Bio-investigación S.L., I+D, León, Spain

Backgrounds

Decline symptoms in young vineyards is one of the biggest problems worldwide in the wine industry, being responsible of significant economic losses. These symptoms are caused by fungal pathogens causing grapevine trunk diseases.

To contribute to reduce this problem, our laboratory have isolated and characterised a selection of endophytic and rhizospheric actinobacteria from the root system of young grapevines. These actinobacteria have a potential activity as biocontrol agents (BCAs), being able to reduce fungal infection levels in young pre-treated grapevines. Three selected actinobacteria have been applied in field assays on grafted vines.

Objectives

The main objective of this work is to develop SCAR markers, specific for each selected actinobacteria, in order to detect and quantified their capability to colonize both the rhizosphere and the root system of the treated plants.

Methods

DNA of the selected actinobacteria has been amplified with only one degenerated primer obtaining characteristics electrophoresis band patterns. Unique bands in these polymorphisms were cloned and sequenced. These sequences were subsequently used to look for intergenic regions that are not evolutionarily conserved. Inside this non-conserved regions, a pair of SCAR primers were designed to identify by qPCR each relevant strain.

Conclusions

Specific primers designed allow us an unequivocally detection of the three corresponding BCAs in vine plants. Moreover, the use of this methodology shows a high reliability for the detection of a particular actinobacteria in a complex population of microorganisms.

FEMS7-2206
Biotechnology / Synthetic Biology / Systems Biology - Part II

NOVOSPHINGOBIUM TARDAUGENS NBRC 16725 AS A NOVEL BIOCATALYST FOR STEROID BIOTRANSFORMATIONS

J. Ibero[1], *D. Sanz*[1], *B. Galán*[1], *E. Diaz*[1], *J.L. García*[1]
[1]*CIB-CSIC, Environmental Biology, Madrid, Spain*

Backgrounds

Several Gram-positive bacteria, mainly actinobacteria, have been described as efficient tools for developing industrial steroid biotransformation processes whereas the use of Gram-negative bacteria in this matter is very limited. Using Gram-negative bacteria would largely facilitate existing bioprocesses due to its easier handling. *Novosphingobium tardaugens* NBRC 16725 (described as strain ARI-1) is a Gram-negative aerobic bacteria isolated from activated sludge in a wastewater treatment plant using 17β-estradiol (an environmentally relevant endocrine disruptor) as sole source of carbon and energy[1]. Recently our group has used this bacterium to unravel the degradation pathway of the 17β-estradiol androgen and to study the metabolic versatility of the strain in steroid biotransformations.

Objectives

To assess the metabolic capabilities of strain NBRC 16725 in order to develop a new bacterial platform for steroid biotransformations.

Methods

A comparative *in silico* analysis was carried out to annotate the genes of strain NBRC 16725 putatively involved in steroid catabolism. Gene expression experiments combined with directed mutagenesis studies were performed to assign specific functions to those genes previously identified. LCMS technique served to characterize intermediate metabolites detected.

Conclusions

The data presented enabled us to expand our knowledge on the metabolic pathways and biotransformation capabilities of a Gram-negative bacterium that becomes a new model system in the steroid field. Moreover, *N. tardaugens* NBRC 16725 might cover a wide spectrum of steroid biotransformation reactions and its improvement as an efficient biocatalyst may lead to promising alternative biotechnological processes.

1.- Fujii *et al*. AEM 68: 2057. 2002

This work has been supported by the Ramón Areces Foundation.

FEMS7-1671
Biotechnology / Synthetic Biology / Systems Biology - Part II

NITRIFICATION WITH SYNTHETIC MICROBIAL COMMUNITIES IN BIOREGENERATIVE LIFE SUPPORT SYSTEMS

C. Ilgrande[1], L. Baptiste[2], W. Ruddy[2], S. Vlaeminck[1,3], N. Boon[1], P. Clauwaert[1]
[1]Faculty of Bioscience Engineering - Ghent University,
Center for Microbial Ecology and Technology CMET, Ghent, Belgium
[2]Institute for Biosciences- Proteomic and Microbiology,
Interdisciplinary Center of Mass Spectrometry CISMa, Mons, Belgium
[3]Research Group of Sustainable Energy- Air and Water Technology,
Department of Bioscience Engineering, Antwerpen, Belgium

Backgrounds

Food and water supply in both short and long-term space exploration missions is restricted by technical and economic constraints. The mass requirements in a Life Support System without recycling is estimated between 5500 and12800 kg per person per year, with a resupply cost of $12600 per kilo.

In order to enable long-term space exploration missions or permanent space habitation, the MELiSSA concept was developed by the European Space Agency (ESA): a bio-regenerative life support system for the complete recycling of gas, liquid and solid waste. The urine production represents a major flux of nutrients in such a life support system but requires further processing in order to make the nutrients available for food production.

Objectives

The Urine NItrification ConsortiUM (UNICUM) is a urine nitrification system designed to produce a nitrate rich fertilizer stream.

Methods

Given the space requirements of biosafety, stability and controllability, all the microorganisms involved in the biological treatment of urine must be known and characterized. As a result, a synthetic approach was favored and *de novo* synthetic communities with different combination of the nitrifying strains *Nitrosomonas ureae* Nm10, *Nitrosomonas europaea* ATCC 19718, *Nitrobacter winogradskyi* Nb-255 and *Nitrobacter vulgaris* Z were assembled. Ureolysis, nitritation and nitratation activity of the different consortia were elucidated in batch. The combinations of *Ns. europaea* and *Nb. winogradskyi* showed a higher metabolic activity, and was selected to evaluate the synergistic interactions by means of proteomic analysis under salinity stress.

Conclusions

Our results demonstrates the feasibility of nitrogen recovery with synthetic communities for future space missions.

FEMS7-2483
Biotechnology / Synthetic Biology / Systems Biology - Part II

EXPLORING THE ROLE OF RESIDUES INVOLVED IN THE OXYANION HOLE OF RHODOCOCCUS SP. CR53 LIPR BY RATIONAL EVOLUTION LIPASES VARIANTS

B. Infanzón[1], *J. Martínez*[1], *F.I.J. Pastor*[1], *P. Diaz*[1]
[1]*University of Barcelona, Department of Genetics- Microbiology and Statistics, Barcelona, Spain*

Backgrounds

Rhodococcus sp CR-53 LipR constitute the first characterized member of the new bacteria lipase family X (Bassegoda A, 2012), and displays an uncommon Y-type class of oxyanion hole shared by members of *C. antartica*, all fungal-lipases (Widmann P, 2010).

Objectives

We aimed to understand the role of residues involved in the oxyanion hole of LipR by the performance of two residues modifications.

Methods

Saturation mutagenesis was used to generate enzyme variants of the specific residues Tyr^{110} and Asp^{111}, previously described for CAL-A as the most relevant amino acids, involved in stabilization of the tetrahedral intermediate. 3D model analysis and *in silico* docking studies were created.

Conclusions

No variants with activity were obtained. However, the LipR D111G variant showed a shift towards longer fatty acid chain-length acyglycerol esters. In addition, in silico models showed that the substitution of Asp by a Gly produces a wider entrance tunnel that could allow for a better and tight accommodation of larger substrates.

FEMS7-0870
Biotechnology / Synthetic Biology / Systems Biology - Part II

GENETIC ENGINEERING OF SACCHAROMYCES CEREVISIAE FOR HYALURONIC ACID PRODUCTION

P. Jeeva[1], G. Jayaraman[1], M. Toivari[2], O. Koivistoinen[2], L. Ruohonen[3]
[1]Indian Institute of Technology Madras, Biotechnology, Chennai, India
[2]VTT Technical Research Centre of Finland Ltd, Metabolic Engineering, Espoo-02044, Finland
[3]VTT Technical Research Centre of Finland Ltd, Industrial biotechnology and food solutions, Espoo-02044, Finland

Backgrounds

Hyaluronan (HA) is a commercially valuable polysaccharide used for various applications. HA is synthesized by polymerization of two nucleotide sugars UDP-glucuronic acid and UDP-N-acetylglucosamine by the polymerizing enzyme HA synthase (*hasA*). Two major genes required for the formation of HA monomers are *hasB* (UDP-glucose dehydrogenase) and *hasD* (glucosamine-1-phosphate acetyl transferase) respectively.

Saccharomyces cerevisiae, a eukaryote, an attractive host for HA production due to its advantages such as lack of pathogenecity and hyaluronidase. In addition, it does not produce HA or any other polysaccharide that contains glucuronic acid due to absence of *hasA* and *hasB.* This makes it a superior model system to understand HA biosynthesis better. Jeong et.al.,(2014) reported HA production in *Pichia pastoris* using *Xenopus laevis has* genes. Hence, *Xenopus has* genes were chosen to engineer *Saccharomyces* for HA production.

Objectives

To metabolically engineer *Saccharomyces cerevisiae* for HA production

Methods

Recombinants were constructed by *MoClo* and CRISPR CAS9 method. HA production was measured using CTAB assay

Conclusions

Initially, the recombinant *Saccharomyces* was constructed by cloning codon optimized *Xenopus hasA* and *hasB* genes in a custom made vector by *MoClo* method. A reduction in their expression levels was observed in co-transformed clones. This was hypothesized to be due to dual high copy number plasmids. To overcome this issue, chromosomal integration of *hasAB* from *Xenopus* and *hasCD* from *Saccharomyces* using CRISPR CAS9 system was carried out. However, strains with *hasABCD* also showed lower expression as compared to strains with only *hasAB*/*hasCD.*

FEMS7-2369
Biotechnology / Synthetic Biology / Systems Biology - Part II

ENGINEERING SYNTHETIC SCAFFOLD TO CO-LOCALIZE PATHWAY ENZYMES FOR HYALURONIC ACID PRODUCTION IN LACTOCOCCUS LACTIS

A.B. Muthukrishnan[1], P. Jeeva[1], V. Devi[1], G. Jayaraman[1]
[1]Indian Institute of Technology IIT Madras, Biotechnology, Chennai, India

Backgrounds

Hyaluronic acid (HA) is a biopolymer synthesized by certain virulent bacteria. The properties of HA offer several biomedical applications. Hence, HA producing avirulent recombinant organisms are being developed. However, challenges such as low yield and low molecular weight still remain. Recent studies suggest that Synthetic scaffolds can be built to target enzymes into spatial proximity for enabling optimal flow of substrates and products. Here we aim to employ this Scaffolding strategy to improve the yield and molecular weight of HA production in *Lactococcus lactis*.

Objectives

Engineering Synthetic scaffolds of pathway enzymes for enhancing the spatial proximity of the polymer precursors for HA synthesis in *Lactococcus lactis.*

Methods

Based on the computational analysis and experimental studies, we've engineered a pNZ8148 vector based plasmid for the expression of a synthetic scaffold to co-localize the HA-precursor synthesis genes (*hasB* and *hasC*) in different ratio. Also, these enzymes are expressed under two different promoters (P_{lacA} and P_{Zn}) enabling modular control over the metabolic flux. The enzyme catalysing the polymerisation (HasA), being a membrane-bound enzyme is integrated into the *L. lactis* chromosome.

Conclusions

The scaffolding strategy using synthetic biology approach employed in this study might allow co-localization of the pathway enzymes (Has B and Has C) in the *L. lactis* strain. Further molecular and metabolic flux analysis studies of this strain will aid in optimizing the ratio of the precursors and the process strategies for improved HA production in *L. lactis*. Insights from this study will contribute further to our understanding of synthetic engineering for metabolic pathways.

FEMS7-0945
Biotechnology / Synthetic Biology / Systems Biology - Part II

THE ENHANCEMENT OF MICROBIAL SYSTEM STABILITY USING A HELPER MICROORGANISM

S.Y. Jeong[1], *T.G. Kim*[1]
[1]*Pusan National University, Microbiology, Busan, Republic of Korea*

Backgrounds

Methanotrophs (aerobic methane oxidizing bacteria) are a biological resource for various purposes, e.g., removal of contaminants and methane. A number of nonmethanotrophic bacteria, such as *Rhizobium, Sinorhizobium,* and *Mesorhizobium*, have been shown to stimulate the growth of methanotrophs when they grow together.

Objectives

This study investigated effects of the *Hyphomicrobium* sp. NM3 as a nonmethanotroph on growth and activity of *Methylocystis* sp. M6 and *Methylosinus trichosporium* OB3b.

Methods

M6 or OB3b was mixed with NM3, isolated from a methanotrophic consortium, at different mixing ratios (1:4~1:14). The consortia at 1:14 were continuously operated at hydraulic retention times (HRT) of 15 and 10 days.

Conclusions

First of all, we found that NM3 stimulated the methanotrophic activity of both M6 and OB3b, which was density-dependent. When a co-culture of M6 and NM3 was cultivated at a HRT of 15 days, the methane removal rate and cell weight were 30.4 ± 4.3 $\mu mol \cdot L^{-1} \cdot d^{-1}$ and 3.0 ± 0.3 $g \cdot L^{-1}$, respectively, in the co-culture while 16.8 ± 4.5 $\mu mol \cdot L^{-1} \cdot d^{-1}$ and 2.1 ± 0.2 $g \cdot L^{-1}$, respectively, in M6 only. In another experiment, the co-culture was operated at a higher dilution rate (a 10 day HRT) for 30 days, with NM3 being spiked when methane removal rate dropped below 20.0 $\mu mol \cdot L^{-1} \cdot d^{-1}$. NM3 spiking immediately recovered the methanotrophic activity of M6. The methane removal and cell weight were 25.8 ± 7.5 $\mu mol \cdot L^{-1} \cdot d^{-1}$ and 2.2 ± 0.2 $g \cdot L^{-1}$, respectively. To sum up, *Hyphomicrobium* sp. NM3 can be a promising biological agent for methane oxidation enhancement in methanotrophic biotechnological processes.

FEMS7-0474
Biotechnology / Synthetic Biology / Systems Biology - Part II

REVEALING THE IMPACT OF HYALURONAN SYNTHESIS ON CELL GROWTH OF STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS

L. Franke[1], Z. Černý[1], D. Čožíková[2], J. Kulhánek[2], A. Růžičková[1], V. Velebný[3], J. Jílková[1]
[1]Contipro a.s., Fermentation group, Dolní Dobrouč, Czech Republic
[2]Contipro a.s., Department of Pharmacokinetics, Dolní Dobrouč, Czech Republic
[3]Contipro a.s., R&D, Dolní Dobrouč, Czech Republic

Backgrounds

Hyaluronic acid (HA) is a linear glycosaminoglycan which is applied in cosmetic and pharmaceutical industry. In the last years HA production is predominantly based on *Streptococci* fermentation. Biosynthesis of HA and cell wall components compete for the same precursors (UDP-N-Acetylglucosamin and UDP-glucuronic acid) in hyaluronic acid producing bacterium *Streptococcus equi* subsp. *zooepidemicus* (SEZ). This interconnection can complicate further bioprocess optimization. Therefore, understanding of this issue is essential for efficient development of new production strains.

Objectives

To quantify the impact of HA synthesis on cell growth, energy consumption and by-products formation in SEZ strain with blocked HA synthesis pathway.

Methods

Hyaluronan synthase deletion mutant (SEZ *ΔhasA)* was prepared by site-directed mutagenesis with the use of thermosensitive plasmid pTE10t. Fermentations were carried out in bioreactors Multifors (Infors HT, Switzerland) with working volume of 800ml. High performance liquid chromatography was performed for organic acids and intracellular UDP-sugars determination.

Conclusions

It was proved that hyaluronan biosynthesis in SEZ represents a substantial energy load. Production of major fermentation products was about 17% lower in case of strain SEZ *ΔhasA*. Concentration of intracellular UDP-sugars was significantly higher due to HA synthesis pathway disruption. Only negligible proportion of these precursors was utilized for cell wall synthesis. Obviously the competing processes of cell wall biosynthesis and HA production do not mean a crucial issue in SEZ strain used for HA production.

FEMS7-0502
Biotechnology / Synthetic Biology / Systems Biology - Part II

BACTERIAL BIOFERTILIZERS DESIGN TO IMPROVE VETCH YIELD PRODUCTION IN CEREAL CROPS ROTATION

J.D. Flores-Félix[1], A. Jiménez-Gómez[1], M. Marcos-García[1], P.F. Mateos[1], E. Martínez-Molina[1], E. Velázquez[1], R. Rivas[1]
[1]University of Salamanca, Department of Microbiology and Genetics, Salamanca, Spain

Backgrounds

Legumes-cereals crop rotation is one of the traditional agricultural practices which has been recovered by the new Common Agricultural Policy. In this case, legumes fix nitrogen due to their symbiosis with nitrogen fixing bacteria and part of this assimilable nitrogen remains in the soil, available for the cereals which are used in rotation. Vetch is one of the most used legume crops in this practise. In addition, to the date, there are no relevant research projects that had analysed the most effective bacterial associations with this kind of crop.

Objectives

The principal objectives of this work were the isolation, selection and characterization of specific bacterial strains suitable to design effective biofertilizers capable to establish an efficient vetch-bacterial symbiosis and therefore the vetch and wheat yields in the crop rotation.

Methods

We identified the 86 isolated strains as belonging to the genus *Rhizobium*. All the strains were able to colonize vetch and wheat roots. Fresh weight of the aerial part of inoculated vetch plants was higher than that of the negative control plants, with values between 75% and 97% for the different strain with respect to the control. According to *in vitro* test, the strain VSAT32 was selected to be inoculated in field trials, which showed higher productions in both crops (120% in vetch and 22% in wheat respect to the control).

Conclusions

Our results showed that strain VSAT32 has the ability to colonise vetch and wheat root surfaces and promote plant growth, suggesting that its interaction with theses crops would be beneficial, being a potential candidate as inoculant for legume-cereal crop rotation.

FEMS7-1042
Biotechnology / Synthetic Biology / Systems Biology - Part II

HYPERSENSITIVITY TO YEAST CWI PATHWAY ACTIVATION THROUGH A SYNTHETIC FEEDBACK LOOP CIRCUIT

E. Jiménez-Gutiérrez[1], E. Alonso-Rodríguez[1], M. Molina[1], H. Martín[1]
[1]Universidad Complutense de Madrid, Departamento de Microbiología II, Madrid, Spain

Backgrounds

The cell wall integrity pathway (CWI) in *Saccharomyces cerevisiae*, mediated by the Mitogen-Activated Protein Kinase (MAPK) Slt2, is responsible for the maintenance of the stability of this essential structure. The activation of this pathway is triggered by cell wall alterations, resulting in the phosphorylation of a MAPK cascade that leads to an intense transcriptional response. Therefore, a precise regulation of the pathway to ensure a proper signaling duration and intensity is essential for an adequate physiological output.

Objectives

Our main goal was to explore the consequences of introducing a positive feedback loop in the CWI pathway, and to analyze the potential of this genetic circuit as a tool for the identification of novel stimuli and regulatory components.

Methods

By using synthetic biology approaches, we have generated a genetic circuit named 'IPAC' (Integrity Pathway Activation Circuit) that provides hyperactivation of the pathway under stimulating conditions. To this end, the *MKK1^{S386P}* allele, coding for a constitutively active version of the CWI MAPKK, was placed under the control of the *MLP1* promoter, which is strongly induced under stimulating conditions.

Conclusions

Stimulation of the pathway in cells containing the IPAC circuit leads to a hyperactivation of the CWI MAPK Slt2, which results in cellular lethality. We have found several mutants showing resistance to the IPAC-induced sensitivity to Congo red, zymolyase and SDS, suggesting that the corresponding genes are involved in the regulation of the CWI pathway. We have also identified novel activating compounds, such as neomycin sulfate, EDTA, LiCl or diphenhydramine hydrochloride.

FEMS7-0588
Biotechnology / Synthetic Biology / Systems Biology - Part II

MECHANISM OF PALLADIUM AND GOLD UPTAKE AND TRANSPORT IN E. COLI

N. Joudeh[1]
[1]*University of Oslo, Department of Biosciences, Oslo, Norway*

Backgrounds

Bacteria use certain heavy metals in their systems, such as copper and nickel, which participate in many cellular processes. They have evolved special machineries to take up and transport these elements. Some bacteria were found to take up other precious heavy elements such as gold and palladium, which are toxic and not needed for metabolism. However, the reason for that and the mechanism of uptake/transport of these elements are still unknown.

Objectives

The biological pathway(s) of Pd and Au uptake and transport will be elucidated. Computational modelling will be used to understand these complex pathways. Later, we will study the ability to manipulate this process genetically to make the bacteria take up larger amounts of elements to be used in future application, such as bioremediation.

Methods

The proteins involved in this process will be studied by characterizing transposon mutants found to affect both the uptake and the transport processes. Then, mutations leading to either reduced or increased uptake will be sequenced, and related genes in the same pathway will be assayed for their involvement in this process. Electron microscopy and energy-dispersive X-ray spectroscopy will be used to characterize the changes in subcellular location of elements due to the introduced mutations.

Conclusions

A debate whether this process is biological or not has been going for many years. Our recent results showed that this process is largely biological. Therefore, we can move to the next step and start to find out the proteins and pathways involved.

FEMS7-2504
Biotechnology / Synthetic Biology / Systems Biology - Part II

LOCAL AND SYSTEMIC IMMUNE RESPONSE TRIGGERED BY MYCOBACTERIA IN BLADDER CANCER TREATMENT

E. Noguera-Ortega[1], R.M. Rabanal[2], E. Gómez-Mora[3], C. Cabrera[3], M. Luquin[1], E. Julián[1]
[1]Universitat Autònoma de Barcelona, Dept. Genètica i Microbiologia, Bellaterra, Spain
[2]Universitat Autònoma de Barcelona,
Unitat de Patologia Murina i Comparada- Departament de Medicina i Cirurgia Animals- Facultat de Ve terinària, Bellaterra, Spain
[3]Hospital Germans Trias i Pujol,
Fundació IrsiCaixa- Institut de Recerca en Ciències de la Salut Germans Trias i Pujol IGTP, Badalona- Barcelona, Spain

Backgrounds

A proper activation of the immune system is a clue step for the efficacy of intravesical instillation of *Mycobacterium bovis* BCG in non-muscle invasive bladder cancer patients. The efficacy of this therapy, in terms of safety and/or survival rates, can be improved by using live or gamma-irradiated non-pathogenic mycobacteria such as *Mycobacterium brumae*, and/or by using new delivery vehicles for mycobacteria such as olive-oil-in water emulsions. At present, however, is it not known to what extend these new therapeutic options modify the induced immune response.

Objectives

We aimed to analyse the local and systemic immune response induced by alternative therapeutic options to BCG.:

Methods

Using the orthotopic murine model of bladder cancer different intravesical treatments were carried out: live or gamma-irradiated BCG or *Mycobacterium brumae*, formulated in aqueous solution or in an olive oil-in-water emulsion. In each group of mice the local immune response was measured by analysing the immune cells infiltrated into the bladder cavity using cytometry, and by measuring the presence of cytokines and chemokines in the urine. The systemic immune response generated was measured by evaluating the production of anti-mycobacteria antibodies in mice sera, and by studying the recall response to mycobacteria antigens in splenocytes cultures from treated and non-treated mice,

Conclusions

Taken together our data indicates that all treatments induce an immune response but at different degrees, being mainly significant when live mycobacteria is used and even enhanced when the emulsion formulation is used for intravesical instillation of mycobacteria.

FEMS7-1720
Biotechnology / Synthetic Biology / Systems Biology - Part II

LIGNINOLYTIC MICROORGANISMS ISOLATED FROM COMPOST PILE WITH POTENTIAL FOR THE IMPROVEMENT OF COMPOSTING PROCCESS AND BIOTECHNOLOGICAL APPLICATIONS

M.D.M. Jurado[1], M.J. López[1], F. Suárez-Estrella[1], J.A. López-González[1], J. Moreno[1]
[1]University of Almería, Biology and Geology, Almería, Spain

Backgrounds

The biodegradation of lignin is a key activity during composting due to its involvement in the humification process and the release of nutrients for microorganisms. This polymer binds covalently both to hemicellulose and cellulose making it difficult to biodegrade and**,** therefore**,** it constitutes a limiting stage of composting. Its biodegradation requires an enzymatic system in which several enzymes participate, namely, lignin peroxidase (LiP), laccase, manganese peroxidase (MnP).

Objectives

The aim of this work was to investigate the capacities related to the lignin metabolism from a collection of microorganisms isolated from horticultural plant waste subjected to composting. This was carried out in order to select the more efficient ligninolytic strains that could be applied for composting improvement and in several biotechnological applications as well.

Methods

The selection was performed in two phases. In the first one, 7 qualitative tests related to the metabolism of lignin were applied, since there is currently no single qualitative evidence to conclusively discriminate ligninolytic activity. Accordingly, in the second one, 49 strains (4 bacteria and 45 fungi) were selected and the production of three ligninolytic enzymes: laccase, LiP and MnP were quantified.

Conclusions

As a consequence of these analyses, several strains that could be considered of interest for the improvement of the composting process and for other biotechnological applications were detected. *Rhodotorula glutinis* 4305 was the most efficient strain for the production of laccase and LiP, whereas *Galactomyces geotrichum* 4210 was the maximum producer of MnP.

FEMS7-0120
Biotechnology / Synthetic Biology / Systems Biology - Part II

THE SEARCH FOR SUSTAINABILITY: BIOLOGICAL METHANE UTILIZATION UNCORKED.

M. Kalyuzhnaya[1]
[1]*San Diego State University, Biology, San Diego, USA*

Backgrounds

Climate change consequences pose significant risks to human health, including reductions of freshwater supplies and food. Global warming is driven by the emission of greenhouse gases, mostly CO_2 and methane. Widespread and steady growth of anthropogenic actions (i.e., fossil fuel production, agriculture, landfill use, and municipal wastewater treatment) makes methane not only the major contributor to climate change, but also the primary target for near-term climate regulation.

Objectives

Biological methane conversion is the main mechanism that controls methane emission in nature. However, the concept of using methane-utilizing microbes for improving sustainability of humane-made systems is just beginning to take shape. Our most recent research efforts are focused on developing bio-based approaches to converting scattered sources of methane (stranded natural gas, coal methane and biogas) into value-added chemicals. This presentation will describe a systems-based investigation of central metabolic pathways in *Methylomicrobium alcaliphilum* $20Z^R$, a model methanotroph and a promising microbial system for methane biocatalysis.

Methods

We combined whole-genome flux-balance modeling with systems-biology approaches (i.e. global metabolomics, transcriptomics and quantitative proteomics) and classical enzymology and genetics to both improve the understanding of methane utilization and facilitate metabolic engineering.

Conclusions

A set of novel mechanisms contributing to the central metabolic pathway regulation and key metabolic precursor formation are proposed. Novel traits allowing enhanced production of biodiesel, commercially important organic acids (i.e. succinic, lactic, gluconic and muconic acids) and amino acids (i.e. ectoine and glutamate) were developed. Our results bridge the potential of biological systems with technology development to address affordable, small-scale methane mitigation.

FEMS7-3219
Biotechnology / Synthetic Biology / Systems Biology - Part II

FUNCTIONAL EXPRESSION AND ENZYMATIC CHARACTERIZATION OF ENDO-ARABINANASES FROM BIFIDOBACTERIA UTILIZING ARABINAN

Y. Kang[1], S.L. Jeong[1], G.H. Nam[1], J.M. Lee[1], N.S. Han[1], T.J. Kim[1]
[1]Graduate School of Chungbuk National University,
Division of Animal- Horticultural and Food Sciences, Cheongju 28644, Republic of Korea

Backgrounds

endo-(1,5)-α-L-Arabinanase (ABN; EC 3.2.1.99) is an *endo*-acting hydrolase which cleaves the internal α-(1,5)-L-arabinofuranosidic linkages of arabinan polymers. According to their substrate preferences and modes of action, ABNs can be categorized into two different groups: (1) ABN-A exclusively hydrolyzes debranched arabinan, (2) ABN-B can hydrolyze α-(1,5)-L-arabinofuranosyl backbone of both sugar beet (branched) and debranched arabinans.

Objectives

Novel *endo*-(1,5)-α-L-Arabinanase genes were found from the genomes of *Bifidobacterium dentium* and *Bf. longum*, respectively. On the basis of the enzymatic properties, each enzyme can be categorized and utilized for the branched arabinooligosaccharides.

Methods

Two putative genes encoding BfdABN-B and BflABN-B were cloned from *Bifidobacterium dentium* ATCC 27679 and *Bf. longum* DSM 20211, respectively. Each gene was constitutively expressed in *E. coli*. Their enzymatic properties were determined.

Conclusions

In the present study, At the amino acid sequence level, they share less than 30% of identities with various known ABNs type-B. Each ABN-B gene, which is fused with six-histidines at C-terminus, was constitutively expressed in *Escherichia coli*. Although the debranched (linear) arabinan is the most preferred substrate, BfdABN-B and BflABN-B possess the significant activity towards sugar beet arabinan as well. While they hydrolyze debranched arabinan into arabinobiose and arabinotriose, both ABN-B enzymes can produce a variety of branched arabinooligosaccharides from sugar beet arabinan. These hydrolases can play an important role in arabinan utilization.

FEMS7-0849
Biotechnology / Synthetic Biology / Systems Biology - Part II

PHENOTYPIC SCREENING OF ESCHERICHIA COLI MUTANTS EXHIBITING ALTERED TRANSCRIPTIONAL PROFILES

Z. Kapustina[1], *G. Alzbutas*[1], *A. Lubys*[1]
[1]*Thermo Fisher Scientific, R&D, Vilnius, Lithuania*

Backgrounds

Gene expression patterns are intimately linked to the physiological state of the cell, which is mirrored in global cellular parameters such as growth rate, cell size, abundance of RNA polymerases and ribosomes. Transcriptional analyses revealed that growth environment as well as metabolic stress caused by overproduction of heterologous proteins is associated with substantial changes in global *E. coli* gene expression profile. However, the effect of genome-wide modulation of transcription patterns on physiological state of *E. coli* cells has not been studied in depth; moreover, analyses are often confined to the knockout mutations.

Objectives

Here we will present the results of a genome-wide transposon mutagenesis study designed to not only disrupt the genes at insertion sites but also significantly enhance the transcription of nearby genomic loci. We aim to identify *E. coli* mutants exhibiting elevated growth rates and/or enhanced efficiency of heterologous protein expression.

Methods

Tn5 minitransposon was designed to contain outward-facing strong promoter sequences. *In vivo* mutagenesis strategy based on delivery of transposition system elements on suicide vectors was developed and used for generation of more than 50 000 insertional mutants. HITS (High-throughput Insertion Tracking by deep Sequencing) technology was optimized for Ion Torrent™ sequencing platform and used for high-throughput analysis of transposon insertion sites after phenotypic screening experiments.

Conclusions

Genetic circuits are coupled to the physiological state of the cell. Alterations of global gene expression profile can lead to identification of intriguing *E. coli* traits leading to the construction of novel cloning and gene expression host strains.

FEMS7-0972
Biotechnology / Synthetic Biology / Systems Biology - Part II

ANTIMICROBIAL EFFECT OF COMPONENTS OF ROOT EXTRACT OBTAINED FROM VEXIBIA ALOPECUROIDES

T. Karpenyuk[1], A. Goncharova[1], T. Kustova[1], M. Zavorotnaya[1], A. Platayeva[1], M. Kucherbayeva[1]
[1]al-Farabi Kazakh National university, Research Institute of Issues in Biology and Biotechnology, Almaty, Kazakhstan

Backgrounds

An actual problem of modern medicine and pharmacology is not only the chemical synthesis of antimicrobial agents, but also the search for new sources of natural antibiotics, including plant origin. Antimicrobial activity of plants, extracts isolated from them and individual components is largely caused by the presence of certain chemical groups belonging to different classes of biologically active substances (BAS).

Objectives

We investigated the antimicrobial properties of the total extract of the roots of plants *Vexibia alopecuroides* and identified BAS causing this activity.

Methods

The total extract was obtained by a two-step maceration fractionated by flash chromatography by appliance Biotage Isoler. Identification of components conducted by high performance liquid chromatography-mass spectrometry and nuclear magnetic resonance, the antimicrobial activity was determined by serial dilutions in broth.

Conclusions

The antimicrobial activity against *Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, Pseudomonas aeruginosa, Candida glabrata, Candida krusei, Candida albicans* was identified for 9 individual compounds belonging to the group of flavonoids. They are identified as the soforaflavon G, leahianon A, alopekuron A, alopekuron B, alopekuron C, alopekuron F, alopekuron D, soforaflavon I and glabrol. For leahianona A, alopekurona A, B and C, the minimum concentration causing 50% of growth inhibition for *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* was <0.8 µg / ml, which is comparable to the minimum concentration of antibiotic Ciprofloxacin, triggering a similar effect.

FEMS7-1903
Biotechnology / Synthetic Biology / Systems Biology - Part II

ISOLATION, PURIFICATION AND ANTIBACTERIAL PROPERTIES OF HUMAN RECOMBINANT LACTOFERRIN FROM TRANSGENIC GOATS

A. Kastsianevich[1], M. Kapustin[1], U. Falkouskaya[1], E. Kolomiets[2], A. Budzevich[3]
[1]Institute of Microbiology of National Academy of Sciences of Belarus, laboratory of protein, Minsk, Belarus
[2]Institute of Microbiology of National Academy of Sciences of Belarus, Laboratory of biological control agents, Minsk, Belarus
[3]Scientific and Practical Center of the National Academy of Sciences of Belarus on Animal husbandry, laboratory of animal biotechnology, Zhodino, Belarus

Backgrounds

Lactoferrin (LF) is iron binding mammalian glycoprotein referred to transferrin family. LF possesses anti-bacterial, anti-oxidant and anti-carcinogenic properties and acts as an iron transfer agent in human body. Due to these reasons, LF is used in a variety of commercial products including functional foods, therapeutic drinks, fermented milk, chewing gum, cosmetics and tooth paste.

Now human LF is substitute by the analog derived from cow milk. It should be noted that protein of animal origin is distinguished from human LF by potential allergenicity and low affinity to human receptors.

Numerous forms of recombinant human lactoferrin (rhLF) have been produced in multiple expression systems, including transgenic plants and animals. Only a few technologies could be scaled up to industrial level. These lactoferrins are rhLF from *Aspergillus awamori*, produced by Agennix (Houston, TX, USA), rhLF from rice and rhLF from goats (Scientific and Practical Center of the National Academy of Sciences of Belarus on Animal husbandry, Belarus)

Objectives

To isolate, purify rhLF from milk of transgenic goats and study its antibacterial properties.

Methods

Centrifugation, spectrometry, SDS-PAGE, ion-exchange chromatography, agar well diffusion method

Conclusions

The lab-scale method of purifying the rhLf from the milk was achieved using ion-exchange chromatography and resulted in 95% purity. The purity of rhLf was determinated by SDS-PAGE.

Strains of Escherichia coli were chosen as test cultures for evalution of antimicrobial activity. Antibiotic kanamycin served as the control. Antimicrobial activity was cheeked via diameter of growth inhibition zones of test cultures. It was found that minimal inhibitory concentration of LF was 50 mg/ml

FEMS7-1855
Biotechnology / Synthetic Biology / Systems Biology - Part II

IRON OXIDIZERS AT HIGH CHLORIDE CONCENTRATIONS: ADAPTATION OF FERRIMICROBIUM ACIDIPHILUM T23T AND ISOLATION OF NOVEL SALT-TOLERANT ALICYCLOBACILLUS STRAINS

M. Kaszuba[1], D. Huynh[1], J. Rivera-Araya[2], S.R. Kaschabek[1], W. Sand[1], G. Levicán[2], M. Schlömann[1]
[1]TU Bergakademie Freiberg, Institute of Biosciences, Freiberg, Germany
[2]Universidad de Santiago de Chile, Departamento de Biología, Santiago de Chile, Chile

Backgrounds

Acidophilic iron oxidizers have been reported to be inhibited by high chloride concentrations. This is problematic for bioleaching operations, since increased chloride concentrations may occur during leaching processes, e.g. the use of sea water or the recycling of the acid solution.

Objectives

Thus, there is a necessity to obtain chloride-tolerant iron oxidizers and a better understanding of biochemical mechanisms enabling them to tolerate high chloride concentrations.

Methods

Ferrimicrobium acidiphilum $T23^T$ was exposed to different chloride concentrations, cultivated in modified DSMZ medium 1190 with 10 mM $FeSO_4$ and 5 mM citric acid in weekly cycles at 30°C, 130 rpm. Adaptation was conducted stepwise with elevated NaCl concentrations. Results showed that *F. acidiphilium* $T23^T$ tolerates ≤ 200 mM NaCl and reproducible subcultivation is achieved in the presence of 100-150 mM NaCl. Reactive oxygen species (ROS) measurements with dichloro-dihydro-fluorescein diacetate exhibited that addition of >100 mM NaCl may cause oxidative stress in bacterial cells. Interestingly, the addition of an osmoprotectant, e.g. 1 mM of trehalose, could lower ROS production and thus reduce oxidative stress. To obtain highly chloride-tolerant bacteria, in addition to those from adaptation studies, bacteria were enriched and isolated from marine tailing samples from Spain on solid medium containing 340 mM NaCl. Acidophiles could successfully be isolated and assigned to the genus *Alicyclobacillus.* The isolates could oxidize ferrous iron in the presence of up to 850 mM NaCl, with iron oxidation capacities being NaCl and temperature dependent.

Conclusions

These findings may be of biotechnological relevance to establish bioleaching of metals at high chloride concentrations.

FEMS7-2803
Biotechnology / Synthetic Biology / Systems Biology - Part II

OVERPRODUCTION OF A TRICHODERMA HARZIANUM CHITINASE AND ANALYSIS OF ITS BIOTECHNOLOGICAL POTENTIAL TO PRODUCE CHITOOLIGOSACCHARIDES

P.E. Kidibule[1], P. Santos-Moriano[2], M. Ramírez-Escudero[3], E. Jiménez[3], D. Rodrigo-Frutos[1], D. Piedrabuena[1], C. Limón[4], M. Remacha[1], J. Sanz-Aparicio[2], F.J. Plou[2], M. Fernández-Lobato[1]
[1]Centro de Biología Molecular Severo Ochoa UAM-CSIC, Virología y Microbiología, MADRID, Spain
[2]Instituto de Catálisis y Petroleoquímica, Biocatálisis Aplicada, Madrid, Spain
[3]Instituto Química Física Rocasolano, Cristalografía y Biología Estructural, Madrid, Spain
[4]Universidad de Sevilla, Genética, Sevilla, Spain

Backgrounds

Chitooligosaccharides (COS) are β-(1,4)-linked oligomers of N-acetyl-glucosamine (GlcNAc) and glucosamine (GlcN) formed by chemical or enzymatic hydrolysis of chitosan or chitin. The growing biotechnological interest of COS in fields such as food or health increases the demand of the producing enzymes as well as their characterization and functional improvement.

Objectives

Express a chitinase of 42 kDa from *Trichoderma harzianum* in a heterologous system, obtain protein levels compatible with its crystallization for the future protein structural resolution and evaluate the ability of the recombinant protein to produce COS.

Methods

The chitinase *gene* cDNA from *T. harzianum* was expressed in *Pichia pastoris* using a restriction-free cloning strategy, production of heterologous protein was analysed and escalated up to a 5 L fermenter level. Recombinant protein was purified and some crystals were obtained which allows undertake the protein structural resolution. Synthesis of oligosaccharides from different substrates were evaluated and optimized using the recombinant enzyme. HPAEC-PAD on a Dionex ICS3000 system and Mass Spectrometry were used in the reaction studies and product characterization.

Conclusions

A chitinase of 42 kDa from *T. harzianum* was overexpressed in *P. pastoris*, the recombinant protein was purified, characterized and crystallized for the protein structural resolution. Production of COS mediated by this enzyme was evaluated and some of the molecules formed were characterized.

FEMS7-2273
Biotechnology / Synthetic Biology / Systems Biology - Part II

STRUCTURAL STABILITY, THERMODYNAMICS AND APPLICATIONS OF A-AMYLASES OF THERMOPHILIC BACTERIA FROM HOT SPRING RESERVOIR IN TULSI SHYAM, GUJARAT (INDIA)

B. Kikani[1], *S. Singh*[2]
[1]*PD Patel Institute of Applied Sciences, Department of Biological Sciences, Changa, India*
[2]*Saurashtra University Campus, UGC-CAS Department of Biosciences, Rajkot, India*

Backgrounds

Thermostable enzymes are industrially very significant, as they possess various benefits over the enzymes of mesophiles. Our research group in India is involved with the distribution, molecular diversity and biocatalytic potentials of thermophilic bacteria and actinomycetes.

Objectives

In the present report, we describe the thermostable α-amylases of thermophilic bacteria. The research mainly dealt with the inherent stability of the amylases.

Methods

The enzymes were purified by a single step purification technique, using hydrophobic interaction chromatography on Phenyl Sepharose 6FF. The α-amylases were characterized with respect to substrate kinetics, stability and structural attributes. Various thermodynamics parameters; ΔS^*, ΔH^*, E and ΔG^* were computed and analyzed to establish the stability of the enzyme-substrate reaction. The changes in the secondary structure of the enzyme under various physicochemical conditions were followed by the Circular Dichroism (CD) spectroscopy. The starch hydrolysis efficiency of the purified enzyme was detected in terms of the dextrose equivalence value.

Conclusions

The overall findings highlighted on the easy and cost effective method of the purification, the intrinsic stability of the amylase as elucidated the CD Spectroscopy and thermodynamic analysis. The calcium independent nature and resistance against chemical and solvent denaturation suggested potential for the commercial applications.

FEMS7-3212
Biotechnology / Synthetic Biology / Systems Biology - Part II

COMPARATIVE GENOME ANALYSES OF BACILLUS STRAINS PRODUCING GAMMA-GLUTAMYLTRANSPEPTIDASE

J.M. Lee[1], J. Lee[1,2], S.L. Jeong[1], Y. Kang[1], S.W. Lee[2], B.S. Hurh[2], T.J. Kim[1]
[1]Graduate School of Chungbuk National University,
Division of Animal- Horticultural and Food Sciences, Cheongju 28644, Republic of Korea
[2]Sempio Foods Company Research Center, -, Cheongju 28156, Republic of Korea

Backgrounds

γ-Glutamyltranspeptidase (GGT) catalyzes the cleavage of γ-glutamyl compounds and the transfer of γ-glutamyl moiety to water, or to amino acid/peptide acceptors. GGT can be utilized for the generation of γ-glutamyl peptides or glutamic acid, which are used as food taste enhancers.

Objectives

In the present study, genome sequence of various *Bacillus* spp. were comparatively analyzed and their enzymatic properties were characterized. These enzymes can enhance the taste of fermented food.

Methods

In the present study, *Bacillus amyloliquefaciens* SMB469 with high GGT activity was isolated from *Doenjang*, a traditional fermented soy food of Korea. The gene encoding GGT from *B. amyloliquefaciens* SMB469 (BaGGT469) was cloned from the isolate, and heterologously expressed in *E. coli* and *B. subtilis*. For comparison, three additional GGT genes were cloned from *B. subtilis* 168, *B. licheniformis* DSM 13, and *B. amyloliquefaciens* FZB42.

Conclusions

The BaGGT469 protein was composed of 591 amino acids. The final protein comprises two separate polypeptide chains of 45.7 and 19.7 kDa, generated via autocatalytic cleavage. It shares about 83.8% and 67.8% of amino acid sequence identities with those from *B. subtilis* and *B. licheniformis*, respectively. These results suggest that BaGGT469 can be utilized for the enzymatic production of various γ-glutamyl compounds. The relationships between their primary structures and enzymatic activities were comparatively investigated on the basis of the genome analyses.

FEMS7-1592
Biotechnology / Synthetic Biology / Systems Biology - Part II

OPTIMISATION OF EXTRACELLULAR LIPASE PRODUCTION BY CRYPTOCOCCUS DIFFLUENS D44

A. Koca[1], S. Durmaz Sam[1], B. Sariyar Akbulut[1]
[1]Marmara University- Faculty of Engineering, Dept. of Bioengineering, ISTANBUL, Turkey

Backgrounds

Lipases don't only catalyse the hydrolysis of triglycerides but also esterification, transesterification, alcoholysis, acidolysis reactions. They are widely used in the food, detergent, and pharmaceutical industry. A wide range of microorganisms produce lipases with remarkably different properties. Increasing the yield of microbial lipases will help diminish production and downstream processing costs. *Cryptococcus diffluen*s D44 is a yeast strain isolated from a petroleum refinery environment with an ability to secrete extracellular lipase enzyme.

Objectives

Medium composition is an important factor that controls the biosynthesis and secretion of lipases. The influence of several edible oils (including olive, sunflower, corn, and sesame oils) as sole carbon sources in the presence and absence of an emulsifying agent (Tween® 80) on the growth and lipase expression of *C. diffluens* D44 cells was investigated in this study.

Methods

Each oil (sterilised by dry heat) was added to sterile growth medium at a concentration of 2.0% (v/v) before inoculation. Growth was monitored via absorbance measurements at 600 nm at pre-determined time intervals. Lipase in the supernatant obtained after centrifugation (at 12,000 rpm for 15 min) was used in enzymatic assays. Lipase activity was determined spectrophotometrically using p-nitrophenyl palmitate as the substrate. Protein measurements were carried out applying Bradford method in which bovine serum albumin was the protein standard.

Conclusions

Depending on our preliminary studies, lipase production increased regularly after lag phase and summitted at the early stationary phase for all oils studied. The highest specific growth rate, biomass production and lipase activity were attained when sunflower oil was the inducer.

FEMS7-1880
Biotechnology / Synthetic Biology / Systems Biology - Part II

DETERMINATION OF NANOPARTICLE BY EXTRACELLULAR SYNTHESIS OF TIO2 BIO-NPS FROM MICROORGANISMS AND ITS BIOLOGICAL ACTIVITY

S.P. Kodal[1], G. Koşarsoy Ağçeli[2], H. Hammamchi[2], N. Cihangir[2], Z. Aksu[1]
[1]Hacettepe University, Chemical Engineering Department, Ankara, Turkey
[2]Hacettepe University, Department of Biology, Ankara, Turkey

Backgrounds

TiO_2 bio-NPs is well known to attack a wide range of microbes by altering the cell membrane structure and its functions. Therefore, nanoparticles have been widely used for the development of many biological and pharmaceutical products.

Objectives

In the present work we report a simple, fast, cost-effective and nonpolluting approach for synthesis of TiO_2 bio-NPs using microorganism species.

Methods

Microorganisms were freshly inoculated in medium and incubated. 25 ml of it was taken and diluted by adding 75 ml of sterile distilled water containing nutrients and allowed to grow for another 24 h, 20 ml of 0.025 M titanium sulfate solution was added to the culture solution and it was heated on steam bath up to 60°C until white deposition starts to appear at the bottom of the flask. After 24 h, the culture solution was observed to have distinctly markable coalescent white clusters deposited at the bottom of the flask. Precipitate obtained was centrifuged and the condensed precipitate was washed with distilled water to obtain neutral pH. The precipitate was calcined at 500°C for 3 h for the removal of biomass organic contents to get TiO_2 NPs. The different parameters were optimized for the synthesized. Characteristics of the synthesized TiO_2 NPs were analyzed using UV–Vis spectrophotometer, SEM, FTIR, XRD, AFM. The efficiency of biologically synthesized TiO_2 NPs was checked for its antibacterial and antibiofilm activity.

Conclusions

The obtained results clearly suggest that microorganisms have potential to synthesis of TiO2 bio-nanoparticles.

FEMS7-1893
Biotechnology / Synthetic Biology / Systems Biology - Part II

BIOSYNTHESIS OF ZIRCONIA NANOPARTICLES BY MICROORGANISMS

S.P. Kodal[1], H. Hammamchi[2], G. Koşarsoy Ağçeli[2], N. Cihangir[2], Z. Aksu[1]
[1]Hacettepe University, Chemical Enginnering Department, Ankara, Turkey
[2]Hacettepe University, Department of Biology, Ankara, Turkey

Backgrounds

Zirconia is an important ceramic with a wide range of applications including electrochemical devices, structural ceramics and catalytic systems. Zirconium oxide nanoparticles powder has been selected to improve the properties, as a bio-compatible material that possesses high fracture resistance, and to improve fracture toughness of ceramics by developing a new generation of ceramic-matrix composites.

Objectives

Biological methods can be used to synthesize zirconia NPs without the use of any harsh, toxic and expensive chemical substances. The aim of this work is to produce zirconia nanoparticles by microorganism which isolated from soil.

Methods

The bacteria were cultured as described elsewhere. After incubation, the culture was centrifuged at 72,000 × g for 20 minutes and washed thoroughly under sterile conditions. The bacterial biomass was then resuspended in 100 ml of a 10^{-3} M aqueous solution of K_2ZrF_6 (pH 3.6) in a 500 ml erlenmeyer flask and kept on a shaker (200 rpm) at 37 ^{o}C. The reaction between the bacterial biomass and ZrF_6^{-2} ions was carried out for a period of 24 h and centrifuged at 72,000 × g for 20 minutes. The characteristics of the synthesized zirconia nanoparticles were analyzed using TEM, FTIR and XRD. The efficiency of biologically synthesized zirconia nanoparticles was evaluated for their antibacterial activity by agar well diffusion method against different pathogenic bacteria as previously described.

Conclusions

Nanoparticles are being synthesized using chemical and physical methods, however the adverse effects of these methods sought for the discovery of novel sustainable methods.

FEMS7-0283
Biotechnology / Synthetic Biology / Systems Biology - Part II

AN EFFICIENT PROCEDURE FOR MARKERLESS DELETIONS OF ANTIBIOTIC CLUSTERS IN ENGINEERED STREPTOMYCES LIVIDANS TK24 STRAIN FOR HETEROLOGOUS EXPRESSION OF SECONDARY METABOLITE GENE CLUSTERS

J. Kormanec[1], *D. Homerova*[1], *R. Novakova*[1], *R. Knirschova*[1], *L. Feckova*[1], *B. Rezuchova*[1], *B. Sevcikova*[1]
[1]*Institute of Molecular Biology Slovak Academy of Sciences, Gene Expression, Bratislava, Slovak Republic*

Backgrounds

The members of the soil bacteria of the genus *Streptomyces* play critical roles in soil ecology and produce various secondary metabolites including many medicinally important antibiotics. *Streptomyces* genomes contain numerous cryptic secondary metabolite gene clusters of unknown functions. An strategy to access these compounds comprises their heterologous expression in engineered well-characterised *Streptomyces* host strains [1]. We set out to develop *Streptomyces lividans* TK24 strain as a host for the expression of heterologous gene clusters using previously developed efficient deletion system based on the positive selection of double crossover events with blue pigment producing gene *bpsA* [2].

Objectives

Optimization of the deletion system based on the *bpsA* gene for successive deletions of dominant antibiotic gene clusters in *S. lividans* T24, followed by removing of antibiotic resistance cassette genes.

Methods

Deletions of antibiotic gene clusters in *S. lividans* TK24 was done as described in [2].

Conclusions

1, We optimized deletion system based on the *bpsA* gene. Using the system several gene clusters were successively deleted in *S. lividans* TK24 strain.

2, Production of mithramycin was substantially increased in the engineered *S. lividans* strain after heterologous expression of mithramycin gene cluster.

Acknowledgements. The research leading to these results received funding from the EC 7th FP7 (project No. 613877). This work was co-funded by the Slovak Research and Development Agency under contract No. DO7RP-0037-12. This work was also supported by the Slovak Research and Development Agency under the contract No. APVV-15-0410 and VEGA grant 2/0002/16 from Slovak Academy of Sciences.

References.

1. Zerikly M, Challis GL (2009) *Chembiochem* 10, 625-633

2. Knirschova R, Novakova R, Mingyar E, Bekeova C, Homerova D, Kormanec J (2015) Utilization of a reporter system based on the blue pigment indigoidine biosynthetic gene *bpsA* for detection of promoter activity and deletion of genes in *Streptomyces. J Microbiol Methods* 113, 1-3.

FEMS7-0926
Biotechnology / Synthetic Biology / Systems Biology - Part II

CONSTRUCTION OF A UNIQUE AND INNOVATIVE PLATFORM FOR PRODUCTION OF PROTEINS BASED ON PSYCHROTOLERANT YEAST DEBARYOMYCES MACQUARIENSIS

E. Krajewska[1], M. Wicka-Grochocka[2], M. Wanarska[2], H. Cieśliński[2]
[1]Gdansk University of Technology, Molecular Biotechnology and Microbiology, Gdansk, Poland
[2]Gdansk University of Technology- Faculty of Chemistry, Molecular Biotechnology and Microbiology, Gdansk, Poland

Backgrounds

The increasing demand for proteins showing high activity at low temperatures, entails the development of technologies for their efficient production and purification. Despite the fact, that many expression systems have been developed by now, they are based mainly on mesophilic or thermotolerant bacteria and yeast. Due to high thermolability of cold-active proteins, these systems are not optimal for their efficient production. The expression system based on psychrotolerant yeast as expression host is potentially the best system for the efficient production of enzymes derived from cold-adapted organisms. Yeast combine the advantages of prokaryotic and eukaryotic expression systems such as fast-growing biomass and efficient secretion of proteins outside the cell, thus allowing a pre-purified preparations of the protein. In addition, yeast as an eukaryotic cells are capable of performing posttranslational modifications, and hence it is possible to obtain correctly folded proteins.

Objectives

The aim of this study was the construction of new platform for the production of proteins, based on pre-selected psychrotolerant yeast cells of *Debaryomyces macquariensis* which were transformed with series of expression vectors. In addition, to test the functionality of the new expression system recombinant plasmids containing the gene encoding β-galactosidase active at low temperature were also constructed, enabling the production of this protein in the yeast *Pichia pastoris* and *Debaryomyces macquariensis*.

Methods

Methods included PCR, cloning of DNA fragments, transformation and the gene expression.

Conclusions

Recombinant *D. macquariensis* strains were able to produce cold-active β-galactosidase from *Paracoccus* sp. 32d. The new expression system was constructed and compared to *Pichia pastoris* system.

FEMS7-2509
Biotechnology / Synthetic Biology / Systems Biology - Part II

EXPLORING BIOLOGICAL POTENTIAL OF CYANOBACTERIA FOR BIOFUEL PRODUCTION

G. Kufryk[1]
[1]*Grand Canyon University, CSET, Phoenix, USA*

Backgrounds

Cyanobacteria are a group of photosynthetic prokaryotes that are capable of oxygenic photosynthesis. Some cyanobacteria are naturally capable of producing compounds that can be used as the source of biofuels: hydrogen, isoprene, C-16 and C-18 lipids, ethanol, etc. Many of them can be genetically engineered to improve the yield of bioproducts, and as photoautotrophic organisms, they can be grown quickly and inexpensively which makes this group particularly suitable for bioengineering.

Objectives

Our goal was genetic engineering of cyanobacterial strains for the purpose of increasing the yield of biohydrogen that was generated in their cultures.

Methods

To this end, the native bidirectional hydrogenase was genetically engineered and overexpressed in several strains of cyanobacteria using a series of alternative promoters. Hydrogen producing activity of bioengineered hydrogenase was tested in vitro. Hydrogen production was recorded and evaluated in the cultures of the cyanobacterial mutants.

Conclusions

Genetic engineering of cyanobacteria allowed us to significantly increase their hydrogen-producing capacity without compromising the stability of the mutant strains. A combination of site directed mutagenesis and overexpression of *hoxE* gene in cyanobacteria resulted in 6 to 8 - fold increase of hydrogen evolution, depending upon the choice of parental strain and growth conditions. As the details of molecular regulation of hydrogen production in the cell become increasingly available, new steps are being taken to improve the level of hydrogen production by cyanobacteria. Due to a photoautotrophic nature and low maintenance cost of these microorganisms, they represent the most economical system for biological hydrogen production with a potential for commercial application.

FEMS7-1607
Biotechnology / Synthetic Biology / Systems Biology - Part II

STUDYING THE ROLE OF PENTOSE PHOSPHATE PATHWAY IN XYLOSE METABOLISM AND FERMENTATION IN THE YEAST OGATAEA POLYMORPHA

O. Kurylenko[1], R. Vasylyshyn[1], J. Ruchala[2], K. Dmytruk[1], A. Sibirny[1]
[1]Institute of Cell Biology- NAS of Ukraine, Department of Molecular Genetics and Biotechnology, Lviv, Ukraine
[2]University of Rzeszow, Department of Biotechnology and Microbiology, Rzeszow, Poland

Backgrounds

The pentose-sugar xylose is the second-most abundant monosaccharide in hydrolysates released from lignocellulosic biomass. *Ogataea polymorpha* is one of the most thermotolerant xylose-fermenting yeast species, however, with low efficiency of xylose alcoholic fermentation in the wild-type strains. The functional role of two key enzymes involved in the non-oxidative part of pentose phosphate pathway, namely transketolase and transaldolase, in xylose metabolism and alcoholic fermentation in *O. polymorpha* remained unclear.

Objectives

Screening of a new targets for improvement of xylose utilization during high-temperature xylose alcoholic fermentation in the yeast *O. polymorpha.*

Methods

Standard methods of yeast molecular genetics and biochemistry were used.

Conclusions

O. polymorpha contains both cytosolic transaldolase (*TAL1*) and transketolase (*TKL1*) and their peroxisomal counterparts (genes *DAS1* and *TAL2*, respectively). The deficiency or overexpression of these genes was examined regarding their roles in xylose utilization and fermentation. The *tal1*Δ, *tal2*Δ and *das1*Δ mutants were constructed by gene disruption technique. The conditionally knockout *tkl1*Δ mutant was constructed by replacing the endogenous promoter of *TKL1* gene by regulated *YNR1* promoter of nitrate reductase, repressed by ammonium sulfate as nitrogen source. A significant decrease in xylose-fermenting ability and totally blocked growth on xylose was observed in *tkl1*Δ and *tal1*Δ mutants. Overexpression of *DAS1* gene in *tkl1*Δ mutant led to restoration of growth on xylose, however, only after prolonged lag phase. The ability of xylose utilization in *tal1*Δ strain was easily restored by overexpression of *TAL2* gene. Moreover, overexpression of each of the mentioned genes resulted in improvement of ethanol production from xylose.

FEMS7-0801
Biotechnology / Synthetic Biology / Systems Biology - Part II

CARYOLAN-1-O1, AN ANTIFUNGAL VOLATILE PRODUCED BY STREPTOMYCES SPP., INHIBITED ENDOMEMBRANE SYSTEM OF FUNGI

S.W. Hong[1], G. Cho[2], Y.S. Kwak[1]
[1]Gyeongsang National University, Applied Life Science, Jinju, Republic of Korea
[2]Gyeongsang National University, Plant Medicine, Jinju, Republic of Korea

Backgrounds

Streptomyces spp. has the ability to produce various secondary metabolites that interact with the environment. It also has potential for its role in the discovery of new antibiotics. Some secondary metabolites have been reported as having antibacterial properties.

Objectives

This study attempted to discover new antifungal volatiles from the genus *Streptomyces* in order to understand how it works as an antifungal compound. To date the biological activity of caryolan-1-ol has not yet been investigated.

Methods

To observe the antifungal activity, a mixture of the main components caryolan-1-ol and the unknown sesquiterpene were collected from *Streptomyces* sp. S4-7. The mixture could act as an antifungal agent and had a dose-dependent character. Furthermore, synthetic caryolan-1-ol also inhibited the dose-dependent growth of the fungus. To understand the mode of action of caryolan-1-ol, chemical-genomics profiling assays were performed.

Conclusions

The results *Streptomyces* produced caryolan-1-ol that affected the endomembrane system by working on sphingolipid synthesis, function of the vesicle and served as the key location for antifungal activity.

FEMS7-3070
Biotechnology / Synthetic Biology / Systems Biology - Part II

INDUSTRIAL PRODUCTION OF PSEUDOMONAS CHLORORAPHIS

E. Lagendijk[1], N. van Biezen[1], C. Blad[1], S. de Weert[1]
[1]Koppert BV, R&D Microbiology, Berkel en Rodenrijs, The Netherlands

Backgrounds

Every year approximately 25 % of the worlds crop yield is lost, mainly due to diseases caused by phytopathogenic fungi. Products on the market to fight these diseases are mostly chemicals, harmful for the health of people and the environment. Biological control of plant diseases by beneficial microbes forms an interesting alternative. These so called beneficial bacteria can be used for sustainable crop protection.

Objectives

The rhizosphere bacterium, *Pseudomonas chlororaphis,* is a good root colonizer and produces a wide variety of secondary metabolites with antifungal activity which makes it an interesting potential for biological crop protection.

Methods

To develop a commercial Plant Protection Product (PPP) that can be used in agricultural applications, it is of important to understand the mode of action and growth characteristics of this bacterium. Process and product development starts with the selection of a suitable media and optimized growth conditions in small scale bioreactors. Besides a reasonable biomass yield also metabolites are of importance. During growth a metabolic profile of this strain is gained.

Conclusions

Pseudomonas chlororaphis is a fast growing strain resulting in high yields of approx. 1E10 cells.ml-1 within 24 hrs. During growth the metabolite profile shows at least 4 different antibiotics with antifungal activity.

FEMS7-2469
Biotechnology / Synthetic Biology / Systems Biology - Part II

SINGLE CELL TIME LAPS MICROSCOPY OF ELECTROSTATICALLY MODIFIED SURFACE OF ALIVE BACTERIAL CELLS REVEALS ELECTROSTATIC EFFECTS ON CELL DIVISION AND ADHERENCE

A. Lapanje[1], I. Rybkin[2], T. Rijavec[1], G. Sukhorukov[3], D. Gorin[2]
[1]Jozef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia
[2]Saratov State University, Saratov State University, Saratov, Russia
[3]Queen Mary University of London, Queen Mary University of London, London, United Kingdom

Backgrounds

In general bacterial cells resemble negative surface charge mainly due to the excess of negatively charged moieties of molecules incorporated in membrane and cell wall. Based on our measurement, bacterial cells posses approximately -40mV of surface potential. This can enable deposition of positively charged molecules on their surface resulting reverting surface potential.

Objectives

Such method of surface modification enables using the layer-by-layer method to enclose bacterial cell in a few nanometre thick permeable and strong capsule as well as to modify their surface potential. In the past, this method was applied in many planar and 3D non-living structures. Here we aimed to develop a whole platform of electrostatic modification of surface of alive bacterial cells.

Methods

Tuning the method it enabled us to cover single bacterial cells by different numbers of layers, affecting the surface permeability, or forming aggregates of defined sizes. Using time laps confocal microscopy we observed that we can control growth and division of single bacterial cells as well as the protein expression based on emGFP reporter. Since we can affect surface charge of bacteria we also attached bacteria on inorganic surfaces forming artificial biofilms. Unexpectedly, we observed effects of different mineral surfaces on metabolic activity. The metagenomic analysis shoved modified climax composition of bacterial biofilms after exposure to the environment of surface previously covered by charged bacterial cells.

Conclusions

This approach enables non-genetic modification of bacterial surface and gives a new tool to study cell-cell and cell-surface interaction as well as to develop new carrier based biotechnological processes.

FEMS7-2507
Biotechnology / Synthetic Biology / Systems Biology - Part II

A NEW SYNTHETIC METHOD OF NON-GENETIC MODIFICATION OF ALIVE BACTERIA CELLS FOR REMOTE GUIDING, IMMUNE PROTECTION, GROWTH CONTROL AND MONITORING OF CELLS IN BACTERIAL THERAPIES

A. Lapanje[1], I. Rybkin[2], O. Gusliakova[2], O. Sindeyeva[2], N. Pytayev[2], D. Gorin[2], G. Sukhorukov[3]
[1]Jozef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia
[2]Saratov State University, Saratov State University, Saratov, Russia
[3]Queen Mary University of London, Queen Mary University of London, London, United Kingdom

Backgrounds

Using a top-down engineering approach, the ideal cancer therapy can be envisioned to use tiny programmable 'robot factories' that specifically target tumours, are selectively cytotoxic to cancer cells, are self-propelled, are responsive to external signals, can sense the local environment and are externally detectable. As such by using synthetic biology approach many bacteria can be modified to use them as small robots in treatments. However, there are also many drawbacks amongst the most important can be the host immune response and dangerous therapies due to low control as well as impossible monitoring of bacterial distribution inside the body.

Objectives

To solve these problems we developed non-genetic modification of surface of bacterial cells. The approach was focused to cover epitopes on the cell surfaces and, to incorporate far red fluorescent dies as well as magnetic particles to observe distribution of cells inside the body and guide cells by the magnetic field, respectively.

Methods

The method was based on the electrostatic deposition of polyelectrolytes. Since we can covalently bind far red Cy7 dye to the charged polyelectrolytes and electrostatically attach magnetite nanoparticles we could modify *Escherichia coli* top10 strain to become visible through the body and guided by magnetic fields. We injected these cells in the mouse cardio-vascular system to observe effect of magnetic field on distribution.

Conclusions

Passive distribution of cells resulted in concentration of amount of cells in lungs and livers and use of permanent magnet increased amount of cells in legs. The number of polyelectrolyte layers controlled division time of bacteria.

FEMS7-1568
Biotechnology / Synthetic Biology / Systems Biology - Part II

NOVEL GENETIC TOOLS FOR PSYCHROBACTER SPP.

R. Lasek[1], *M. Krolikowski*[1], *D. Bartosik*[1]
[1]*University of Warsaw- Faculty of Biology, Department of Bacterial Genetics, Warsaw, Poland*

Backgrounds

The study of cold-adapted bacteria is a promising branch of microbiology, especially given their huge biotechnological potential. However, the research on psychrophiles is hindered by the lack of specific genetic tools for this group of prokaryotes. Having studied in detail a pool of Arctic strains of the genus *Psychrobacter*, we decided to use selected genetic modules of their native plasmids in the construction of novel *Psychrobacter*-specific vectors.

Objectives

We aimed to create a series of new genetic tools functional in DAB_AL43B, a well-characterized *Psychrobacter* strain with a completely sequenced genome. It includes (i) a protein expression vector (pExPsy), (ii) a vector for testing promoter activity in vivo (pRSPsy), and (iii) a two-plasmid system for introducing markerless deletions in the DAB_AL43B genome (pLOL/pROFL).

Methods

Standard genetic manipulations were used in the construction of plasmid vectors. The obtained shuttle vectors were introduced into *Psychrobacter* sp. cells via triparental mating. The *E. coli*-derived *lacZ* gene was used as a reporter gene or a counterselection marker.

Conclusions

Several new genetic tools were obtained and verified to be functional in *Psychrobacter* sp. DAB_AL43B. The pExPsy vector carries a strong promoter P_{SLF} induced by sodium dodecyl sulfate and laurylaldehyde, which allows for the inducible expression of His-tagged proteins. The RSPsy vector is used to conduct high throughput LacZ activity assays, whereas in the pLOL/pROFL system the *lacZ* gene serves as a counterselection marker (the product of X-gal hydrolysis being deleterious for DAB_AL43B). We believe that these novel tools will facilitate further studies of *Psychrobacter* spp.

FEMS7-1069
Biotechnology / Synthetic Biology / Systems Biology - Part II

IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS PRESENT IN THE ASSEMBLY LINE OF KALIMANTACIN, A SECONDARY METABOLITE ANTIBIOTIC

T. Lathouwers[1], B. Uytterhoeven[1], C. Michiels[2], R. Lavigne[1]
[1]KU Leuven, Biosystems- Lab of Gene Technology, Leuven, Belgium
[2]KU Leuven, Department of Microbial and Molecular Systems - Centre for Food and Microbial Technology, Leuven, Belgium

Backgrounds

Secondary metabolites are natural compounds produced by bacteria. They are not essential for development of their producers, but provide them with a selective advantage, like elimination of environmental competitors by antimicrobial activity. Two classes of secondary metabolites are the polyketides and non-ribosomal peptides. Although chemical diverse, both groups are produced by large assembly lines. Each assembly line consists of modules, which are built up by multiple enzymatic domains. Every module attaches a new building block to the metabolite's backbone, realizing elongation and functional specialization.

Objectives

Natural hybrid pathways exist, combining both polyketide and non-ribosomal peptide modules. This inspired researchers to create new antibiotics by designing hybrid lines, but often production of metabolites was abolished, possibly due to a lack of protein-protein interactions linking the different modules. In this project, protein interactions were identified in the biosynthesis cluster of kalimantacin, a hybrid antistaphylococcal compound produced by *Pseudomonas fluorescens* BCCM_ID9359.

Methods

In this study, 63 fragments, representing the domains and connective regions of the kalimantacin assembly line, were amplified for yeast two-hybrid analysis. The use of an integrated pool-array technique allowed testing of all different combinations, thereby creating a protein interaction map of the entire biosynthesis cluster. In total, 28 interactions were identified, 13 of them could be confirmed further.

Conclusions

Results include a specific interaction between a *trans*-acting enoyl reductase and carrier proteins of the pathway, also an interaction possibly linking two modules. In future, these interactions are examined further and will be a useful basis for hybrid engineering of clusters.

FEMS7-2020
Biotechnology / Synthetic Biology / Systems Biology - Part II

NORINE, FLORINE, S2M : POWERFUL BIOINFORMATICS RESOURCE AND TOOLS TO SCREEN FOR NOVEL NONRIBOSOMAL PEPTIDES, NATURAL METABOLITES WITH VERSATILE ACTIVITIES

Q. Esmaeel[1], Y. Dufresne[2], A. Flissi[2], M. Pupin[2], P. Jacques[1], V. leclere[3]
[1]Univ Lille-Sciences et technologies, Institut Charles Viollette, villeneuve d'ascq, France
[2]Univ Lille-Sciences et technologies, Laboratoire CRIStAL, villeneuve d'ascq, France
[3]Univ Lille - Sciences et technologies, Institut Charles Viollette, villeneuve d'ascq, France

Backgrounds

Secondary metabolites are a huge resource of natural compounds with applications in different area including medicine and agriculture. Many of them, harbouring a peptidic structure are produced through a ribosome-independent pathway represented by multienzymatic modular complexes working as assembly lines, so-called nonribosomal peptides synthetases. The nonribosomal modular synthesis, together with the monomer recruitment mecanism can drive the production of compounds displaying large structural biodiversity leading to attracting biological activities or properties. Indeed, such nonribosomal peptides are marketed as antibiotics (ie penicillin or vancomycin) or are promising sustainable compounds for pesticide replacement as they can be used as biocontrol agent to protect cultures from fungal diseases.

Objectives

The objective of the work was the development of bioinformatics tools for *in silico* genome mining leading to the accelerated discovery of novel nonribosomal peptides. Our work was especially focused on the identification of siderophores and lipopeptides, for their putative applications in biocontrol.

Methods

Specific bioinformatics tools were developed (http://bioinfo.cristal.univ-lille1.fr/NRP/) and related in Florine workflow. The discovery workflow was applied on genome sequences available in public databases.

Conclusions

The availability of increasing number of genome sequences of microorganisms combined with bioinformatic approaches has led to the discovery of a large number of new NRPs. *In silico* analysis on 48 gapless complete genomes of *Burkholderia* revealed at least 161 clusters potentially producing 11 novel products including siderophores and lipopeptides. Some predictions, supported by experimental data, will be presented. The role of these non ribosomal peptide *in vivo* and potential applications will be discussed.

FEMS7-0817
Biotechnology / Synthetic Biology / Systems Biology - Part II

THE WHOLE GENOME ANALYSIS OF ASPERGILLUS SOJAE SMF 134 ISOLATED FROM MEJU, KOREAN TRADITIONAL FERMENTED SOYBEAN BRICK

I. Lee[1], K.M. Kim[1], Y.H. Choi[2], B.S. Hurh[2]
[1]Kookmin University, Bio Fermentation Convergence Technology, Seoul, Republic of Korea
[2]Sempio Foods Company, Sempio Fermentation Research Center, Chungcheongbukdo, Republic of Korea

Backgrounds

Aspergillus sojae may have a great potential as a starter (*koji*) mold for the soybean fermentation due to its high protease and leucine aminopeptidase (LAP) activities.

Objectives

We analyzed the whole genome of *A. sojae* SMF 134 isolated from *meju*, Korean traditional fermented soybean brick with focus on its biochemical abilities and toxigenecity to evaluate its merits as a starter mold at the genomic level.

Methods

The genomic DNA of SMF 134 was sequenced in the PacBio and analyzed using diverse bioinformatics programs.

Conclusions

The genome size was 40.1 Mbp with 13,748 ORFs. The *A. sojae* SMF 134 had more protease genes (151 genes) than *A. oryzae* (134 genes), which verified higher proteolytic activity of *A. sojae* than that of *A. oryzae*. Especially proteases such as aminopeptidase and carboxypeptidase that play important roles in flavor development and debittering during soybean fermentation were more in *A. sojae* than in *A. oryzae*. In toxigenicity analysis, the *A. sojae* SMF 134 had all orthologs of the aflatoxin (AF) biosynthesis genes, however, it appeared to be non-aflatoxigenic because of a termination point mutation in *aflR* and the lack of the polyketide synthase (PKS) gene. In addition, the *A. sojae* SMF 134 could not produce cyclopiazonic acid (CPA) due to the defective genes in the CPA cluster. The genomic data of *A. sojae* SMF 134 will be used to study *A. sojae* genetically for understanding the production of various enzymes that play a key role in soybean fermentation and characteristics of it as a starter mold.

FEMS7-1714
Biotechnology / Synthetic Biology / Systems Biology - Part II

DEVELOPMENT OF WHOLE CELL HEAVY METAL BIOSENSORS BY RECOMBINING T7 TRANSCRIPTIONAL MODULES AND CADC BIOPARTS

H.J. Kim[1], H. Jeong[2], D.W. Lee[3], S.J. Lee[1]
[1]Chung-Ang University, Department of Systems Biotechnology, Anseong, Republic of Korea
[2]Korea Research Institute of Bioscience and Biotechnology, Infectious Disease, Daejeon, Republic of Korea
[3]Kyungpook National University, School of Applied Biosciences, Daegu, Republic of Korea

Backgrounds

Genome sequencing analysis of *Bacillus oceanisediminis* 2691 revealed redundant heavy metal resistance related genes encoding heavy metal-sensing transcriptional regulators (i.e. *cadC*), and heavy metal efflux pumps.

Objectives

In this study, we attempted to develop whole cell heavy metal biosensors using synthetic genetic circuits with T7 transcriptional modules and CadC bioparts.

Methods

In application aspects, these heavy metal sensing genes could be used as synthetic biological parts for the development of heavy metal-specific whole cell biosensors. Six *cadC* genes were transcriptionally fused with *egfp* gene in *E. coli.* We confirmed that cellular fluorescence was generated differentially in response to various heavy metal ions. These results indicated that CadC proteins could be used as sensory modules for specific heavy metal ions in cells. Next, T7 transcription system was combined with CadC bioparts to generate synthetic genetic circuity to amplify intracellular fluorescent signals, and to upgrade the sensitivities of heavy metal biosensors.

Conclusions

This study shows how to screen sensory and regulatory bio-parts, including CadC regulators and *cadO* operators, from the genome of a novel bacterial isolate from ocean sediment, and how to obtain proper bio-parts with distinct specificities from among reiterated paralogous genes. Furthermore, we combined T7 signal amplification modules with CadC to generate CadC-T7 synthetic circuits to detect heavy metals with enhanced specificity and sensitivity.

FEMS7-1705
Biotechnology / Synthetic Biology / Systems Biology - Part II

FUNGAL GROWTH IN A CHEMOSTAT SHOWS POTENTIAL FOR A ROBUST AND SIMPLE PRODUCTION PLATFORM AT 40°C

S. Barig[1], H. Leupold[1], K.P. Stahmann[1]
[1]Brandenburgische Technische Universität Cottbus-Senftenberg, Technische Mikrobiologie, Senftenberg, Germany

Backgrounds

Microorganisms are used for industrial production since a century, e.g. citric acid production by *Aspergillus niger*, or since decades, e.g. microbial riboflavin production from renewables by the fungus *Ashbya gossypii*, as well as the bacterium *Bacillus subtilis* [1, 2].

Objectives

Two drawbacks of microbial systems are addressed: first, a suboptimal cultivation temperature limiting metabolic flux and causing cost-effective cooling equipment, and second, the need of sterile technique.

Methods

A screening of environmental samples was performed using agar plates containing a defined mineral salts medium with nitrate as sole nitrogen, plant oil as sole carbon source and acidic pH excluding sterile technique [3]. Filamentous fungi were isolated at up to 50°C. Colony growth rate on the agar surface was compared. Fungal isolates were identified by ITS sequencing and investigated in chemostat experiments.

Conclusions

Screening revealed hundreds of fungal strains from different environmental sources. Temperature optima of the best strains were found between 39°C and 42°C. Isolate *Aspergillus fumigatus* AR04 showed maximum growth rate of 1,100 µm/h at 40°C on agar plates with full medium, while *A. gossypii* grew 150 µm/h. *A. fumigatus* AR04 grew even faster than reference strains of *A. fumigatus*, *A. niger*, *A. oryzae*. In chemostat cultivations with minimal medium *A. gossypii* showed at dilution rate of 0.32 h^{-1} and 28°C a biomass production of 0.91 g/L [4]. Steady state was still adjustable for AR04 at 40°C at 0.7 h^{-1} and biomass concentration of 1.2 g/L. This behavior fits to the Arrhenius equation and suggests a growth maximum never reported before for a fungus.

FEMS7-0180
Biotechnology / Synthetic Biology / Systems Biology - Part II

IMMUNOGENICITY AND BIOACTIVITY OF A MYCOPLASMA PNEUMONIAE SUBUNIT VACCINE MIXED WITH VARIOUS ADJUVANTS IN BALB/C MICE

Y. Li[1], J. Guo[2], X. Luo[2], Y. Qi[2], S. Li[2], Y. Pan[2], J. Li[2], T. Xu[2], Q. Yin[2], Y. Shao[2], B. Yang[2]
[1]Huadong Research Institute for Medicine and Biotechniques, Nanjing, China
[2]Huadong Research Institute for Medicine and Biotechniques,
Department for Pharmaeutical biotechnology, Nanjing, China

Backgrounds

Mycoplasma pneumoniae (M.pneumoniae)is a human pathogen that colonizes the mucosal surfaces of the respiratory tract.The patients show flu-like sumptoms but characteristically the infection is chronic in onset and recovery.Pneumonia can be followed by extrapulmonary manifestations affecting mainly are skin and the central nervous system.In addition,the cell wall-less bacterium M.pneumoniae is resistant to common-first-line β-lactam antibiotics.Vaccination is a possibility to effectively reduce the incidence of infection due to M.pneumoniae.In this study,we used the recombinant protein P1C-P116N-P30C(MP559)as antigen in combination with different adjuvants for an optimized immunization procedure and evaluated the antibody response as well as celluar response in an accepted animal model.

Objectives

To identify effective adjuvants, immunity dose and procedure for the mycoplasma pneumoniae(MP) subunit vaccine MP559.

Methods

Groups(n=16) of female 6-week-old BALB/c mice (22±2g) were immunized respectively with MP subunit vaccine MP559 alone (5μg or 20μg dose), MP559 with Alum-adjuvant, MP559 with PolyI:C adjuvant , MP559 with CpG adjuvant, MP559 with Alum-PolyI:C adjuvant, MP559 with Alum-CpG adjuvant, MP559 with PolyI:C-CpG adjuvant, MP559 with Alum-PolyI:C-CpG adjuvant by intramuscular injection four times at a 2-week interval. All animals blood was collected via the sinus of eye on the day before each immunization and the second, third and fourth weeks after last immunization, the sera antibody titers were detected by ELISA. After the final immunization, the spleen cells were prepared and then stimulated with 10μg of MP559 for 72h. IL-1, IL-4 and IFN-γ in the supernatant of cultured splenical cell were detected with ELISA Kits.

Conclusions

Immunizing three times with 20μg MP559 with Alum-PolyI:C adjuvant at a 2-week interval could induce the best humoral and cellular immune responses in BALB/c mice. This study laid the foundation for clinical trial of mycoplasma pneumoniae subunit vaccine MP559.

FEMS7-0796
Biotechnology / Synthetic Biology / Systems Biology - Part II

BIOCHEMICAL CHARACTERIZATION OF SERINE PROTEASE-LIKE ENZYME FROM STREPTOMYCES LAURENTII

S.V. Ganesan[1], B.L. Liu[1], M.J. Lee[1], C.A. Chang[1]
[1]Chaoyang University of Technology, Applied Chemistry, Taichung, Taiwan R.O.C.

Backgrounds

Orchids are vulnerable to several disease caused by mechanically transmissible and highly *in vitro* stable plant viruses such as odontoglossum ringspot virus (ORSV) or cymbidium mosaic virus (CymMV). Previous studied have shown that an actinomycetes isolate can secrete a protease-like substance in its culture filtrate that can degrade coat proteins of ORSV and CymMV and abolish their infectivity.

Objectives

The present study attempts to identified this actinomycetes isolate, and the biochemical characterization of a thermoactive and thermostable serine protease-like enzyme from actinomycetes cultivation filtrate were also investigated.

Methods

The 16S rRNA gene sequences to study bacterial phylogeny and taxonomy was used. The cultivation filtrate was partially purified by ammonia sulfate and preliminary enzymological study were engaged. The protease activity was confirmed by zymogram-native PAGE.

Conclusions

The isolate was identified as *Streptomyces laurentii* N74-3 based on their morphological characters and 16S rDNA sequencing by BLASTN and phylogenetic tree analysis. The predicted RNA secondary structure showed the free loop energy score of -286.60, which were characterized by the formation of several helices and showed more structural motifs. Actinomycete was analysed for the production of serine protease-like enzyme on skim milk casein agar and soybean meal mediums. The protease activity was performed in zymogram, which showed the clear band at 35 kDa. The enzyme showed greater activity at the optimum temperature between 70 and 80°C. Enhanced activity was also observed at alkaline pH 10 to 12. The isolated enzymes were strongly inhibited by PMSF, which suggested that the enzyme may belongs to serine protease enzyme family.

FEMS7-1445
Biotechnology / Synthetic Biology / Systems Biology - Part II

COMPARATIVE PROTEOMICS OF A BUTANOL TOLERANT LACTOBACILLUS MUCOSAE ISOLATE DURING GROWTH WITH 2, 3 AND 4% BUTANOL

S. Liu[1]
[1]*NCAUR, Renewable Product Technology, Peoria, USA*

Backgrounds

Butanol can be naturally produced via microbial fermentation by *Clostridium* species. But the current available butanol fermenting microbes are sensitive to increased butanol content.

Objectives

One strategy to overcome butanol toxicity is to modify *Clostridium* species by introducing butanol tolerance related genes.

Methods

Several *Lactobacilli* were found capable of growth in 3-4% butanol after long term adaptation. One particular isolate, *Lactobacillus mucosae* BR0605-3 showed most robust growth in 4% butanol, was used to identify new butanol tolerance genes by 2D gel analyses and proteomics.

Conclusions

A total of 603 spots analyzed showing differences indicating the global and complex nature of cellular responses to increased butanol in the growth medium. Among 30 proteins identified 18 shown increased expression levels and 12 shown reduced expression levels by the presence of 2%, or 3% or 4% butanol. The *Lactobacillus mucosae* butanol tolerance responses involved general stress responses (GroEL, GroES and DnaK), and the shifts from active carbohydrate metabolisms to energy saving modes, plus adaptation of butanol through a balanced redox potential. Pentose phosphate pathway and some amino acid syntheses were slowed down during growth with increased butanol. The ratio of NAD(P)H /$NAD(P)^+$ was likely increased due to reduced activity of D-lactate dehydrogenase and 6-phospohogluconate dehydrogenase.

FEMS7-0762
Biotechnology / Synthetic Biology / Systems Biology - Part II

APPLICATION OF THE RECOMBINANT CUTINASES ANCUT 3 AND ANCUT 4 OF ASPERGILLUS NIDULANS IN DEGRADATION OF POLYESTERS

A. Llanos Reyes[1], K. Rosete Viveros[1], S. Morales García[1], M. Sánchez Sánchez[1], C. Peña Montes[1], A. Farrés González[1]
[1]Universidad Nacional Autónoma de México, Departamento de Alimentos y Biotecnología, Mexico City, Mexico

Backgrounds

Polyesters excessive consumption in daily life has had a negative impact on the environment. To achieve a faster degradation enzymes have been used and among them, cutinases have been employed successfully to hydrolyze a wide variety of polyesters. In our research group, four cutinases from *Aspergillus nidulans* have been identified, isolated, cloned and expressed in *Pichia pastorias* (Vega, 2013, Bermúdez, 2013 and Rosete, 2017). Of these four cloned enzymes, ANCUT 1 and ANCUT 2 have provided effective results in the degradation of polyesters (Sánchez, 2015 and Morales, 2017).

Objectives

The objective of this study is to evaluate the degradation potential against different polyesters using the ANCUT3 and ANCUT4 recombinant cutinases of *Aspergillus nidulans*, due to their close phylogenetic relationship with ANCUT 1 and ANCUT 2 (Castro, 2014), which suggests that they could also carry out this process.

Methods

The ability of these enzymes in the degradation of polyesters was evaluated using polycaprolactone (PCL), polybutylenesuccinate (PBS), polyethylenesuccinate (PES), polylactic acid (PLLA) and polyethyleneterephthalate (PET). Preliminary assessment showed hydrolysis halos in plates. This was followed by the determination of the optimal substrate degradation conditions: temperature, time, pH and influence of the solvent. The degradation degree was determined by weight loss, absolute acidity and scanning electron microscopy.

Conclusions

The results show that the ANCUT3 and ANCUT4 cutinases are an interesting alternative in the enzymatic degradation of this type of polymers, since the results have been favourable. As future work it is intended to test a mixture of the 4 enzymes to obtain a higher polymer degradation.

FEMS7-1902
Biotechnology / Synthetic Biology / Systems Biology - Part II

QUERCITRIN FUNCTIONALIZED NANOSTRUCTURED BONE IMPLANT TITANIUM SURFACES FOR THE MODULATION OF BACTERIAL AND CELLULAR ADHESION

M.A. Llopis-Grimalt[1], M.A. Forteza-Genestra[1], A.M. Amengual-Tugores[1], J.M. Ramis[1], M. Monjo[1]
[1]Grupo Terapia Celular e Ingeniería Tisular- Institut Universitari d'Investigació en Ciències de la Salut- Universitat de les Illes Balears, Instituto de Investigación Sanitaria de Palma, Palma, Spain

Backgrounds

Titanium implants are widely used in modern medicine with several applications such as orthopedic and dental implants. Titanium implants associated infections are the main cause of implant failure and are usually caused by the patient's microbiota. *Staphylococcus epidermidis* is one of the main causative agents of implant-related infections and its ability to form biofilms plays a key role in this process.
There are several strategies for the development of biomaterials with improved integration of the patient's tissues and reduced bacterial adhesion and biofilm formation. One strategy is the formation of nanostructures, such as ordered nanotube structures or nanopores on the implant surface. Another strategy is their functionalization with active biomolecules in order to make virtual bioinert materials become bioactive. Flavonoids are small polyphenolic molecules with a broad range of bioactivities such as antioxidant, antibacterial and anti-inflammatory properties. Among different flavonoids, quercitrin was selected in previous studies as our best candidate for coating surfaces of dental and orthopedic bone implants.

Objectives

The main objective of this research was the development of quercitrin functionalized nanostructured titanium surfaces that reduce biofilm formation and promote osseointegration with potential application in implantable medical devices.

Methods

Functionalized nanostructured titanium surfaces were developed by electrochemical anodization and evaluated for *Staphylococcus epidermidis* CECT4184 adhesion and biofilm formation by scanning electron microscopy, crystal violet and serial dilution agar plating method. In addition, human Bone Marrow Mesenchymal Stem Cells were used to test cell adhesion.

Conclusions

The obtained results have allowed us to establish the best quercitrin functionalized nanostructure for improved bone implants.

FEMS7-2551
Biotechnology / Synthetic Biology / Systems Biology - Part II

DNA TRANSFER AND GENOMIC INTEGRATION IN HUMAN CELLS USING TYPE IV SECRETION SYSTEMS OF HUMAN PATHOGENS

C. González-Prieto[1], R. Gabriel[2], D.L. Guzmán-Herrador[1], S. Steiner[3], C. Roy[3], M. Schmidt[2], M. Llosa[1]
[1]Universidad De Cantabria, IBBTEC, Santander, Spain
[2]National Center for Tumor Diseases, Department of Translational Oncology, Heidelberg, Germany
[3]Yale University, BCMM, New Haven CT, USA

Backgrounds

- Gene therapy strategies require methods to introduce DNA into specific human cell types and to integrate it in the genome for stable expression.

- We have shown that any DNA can be introduced into specific human cell types as a protein-DNA complex through the Type IV Secretion System (T4SS) of *Bartonella henselae* by a mechanism resembling bacterial conjugation.

Objectives

- To extend this DNA delivery system to T4SS from other human pathogens targeting different cell types

- To follow the fate of the delivered DNA and analyze the integration pattern
- To compare the efficiency of different conjugative systems

Methods

- Infection of human cultured cell lines with human pathogens containing mobilizable plasmids with markers for eukaryotic selection and different conjugative systems.
- Evaluation of transient and permanent expression of introduced DNA by measuring GFP positive cells and neomycin resistant colonies, respectively.

- Determination of the integration pattern of the foreign DNA in the human genome by LAM-PCR and high throughput sequencing.

Conclusions

- We can deliver DNA into different human cell types through the T4SS of Bartonella henselae, Legionella pneumophila, and Coxiella burnetii, suggesting DNA delivery can be accomplished by many, if not all, bacterial T4SS.
- The efficiency of transfer depends on the conjugative relaxase
- The relaxase TrwC promotes genomic integration of the incoming DNA

FEMS7-2816
Biotechnology / Synthetic Biology / Systems Biology - Part II

ORAL COLONIZATION AND PH BUFFERING BY STREPTOCOCCUS DENTISANI, A PROBIOTIC BACTERIUM TO PREVENT TOOTH DECAY

A. López-López[1], A. Mira[1], C. Llena[2], T.I. Nicolescu[2], M.D. Ferrer[1]
[1]Centro Superior de Investigación en Salud Pública, Genomics and Health, Valencia, Spain
[2]Facultad de Medicina y Odontología. Universidad de Valencia, Estomatología, Valencia, Spain

Backgrounds

A new bacterial species, named *Streptococcus dentisani*, was isolated from dental plaque of caries-free individuals. It has a double effect that has been proposed to potentially prevent tooth decay: an antimicrobial activity against oral pathogens and a pH buffering capacity through the arginolytic pathway.

Objectives

The aim of this work was to evaluate the colonization efficiency and pH buffering in a pilot clinical study

Methods

Eleven adult volunteers enrolled in the study. The probiotic was applied at a total dose of 10^9 CFUs, administered in a buccoadhesive gel for 5 minutes, either in a single dose or daily for a week. Dental plaque and saliva samples were collected after 15 and 30 days of first application where amounts of *S. dentisani* and pH was measured by qPCR and reflectometry, respectively.

Conclusions

There was a significant increase in *S. dentisani* cells/ng of plaque DNA and in the percentage of *S. dentisani* from total bacterial cells, at day 15 but not at day 30. In addition, there was a significant increase in basal salivary pH both at day 15 ($p=0.024$) and day 30 ($p=0.014$), and in the salivary pH after a sugar rinse at day 30 ($p=0.029$). The results indicate the *S. dentisani* is transiently able to colonize the oral cavity and to buffer oral pH, suggesting a promising probiotic potential, as dental caries is caused by acid-mediated tooth demineralization. Future randomized, placebo-control clinical trials should evaluate the use of *S. dentisani* to prevent tooth decay.

FEMS7-1908
Biotechnology / Synthetic Biology / Systems Biology - Part II

PRODUCTION OF BIOPLASTICS IN DEFINED MIXED CULTURES OF SYNECHOCOCCUS ELONGATUS AND PSEUDOMONAS PUTIDA

H. Löwe[1], K. Pflüger-Grau[1], A. Kremling[1]
[1]Systems Biotechnology, Mechanical Engineering, Garching, Germany

Backgrounds

Cyanobacteria that have been genetically modified to secrete carbohydrates, can even exceed the areal productivity of the traditional crop-based production of sucrose [1]. Compared to conventional feedstocks, cyanobacteria can also be cultivated on non-arable land and can grow in salty or brackish water, making them an interesting alternative and field of research.

Objectives

Our aim is to couple genetically engineered *Synechococcus elongatus* PCC7942 *cscB*, which releases sucrose into the fermentation broth, to *Pseudomonas putida cscAB* that was genetically modified to consume this sugar.

Methods

A common medium based on BG-11 was designed that supported growth of both organisms. Sugar production was then studied in shaking flasks and a 1.8-L photobioreactor yielding up to 3 g/L of sucrose in the culture supernatant after one week. In a first attempt, sucrose containing culture supernatant was successfully used to support the growth of recombinant *P. putida cscAB*. As next step, both organisms were able to grow simultaneously under photosynthetic conditions, allowing *P. putida* to produce polyhydroxyalkanoates up to 0.1 g/L from cyanobacterial sucrose.

Conclusions

Taken together, cyanobacterial carbohydrates were successfully used as a carbon source for the cultivation of *P. putida*, a potential workhorse for industrial biotechnology. This extensible technology thus offers a potentially cheap feedstock for fermentation of plentiful possible products [2] and might be an opportunity to make industrial biotechnology more cost-competitive in the future.

[1] Ducat et al. (2012) Appl. Environ. Microb. 78(8): 2660-2668

[2] Belda et al. (2016) Environ. Microbiol. 18(10): 3403-3424

FEMS7-3140
Biotechnology / Synthetic Biology / Systems Biology - Part II

CARBOXYL NANOPARTICLE ADHERENCE TO EXTERIOR OF C. ALBICANS HYPHAE CELL WALL IS MEDIATED BY THE HYPHAL ADHESIN ALS3

A. Lyden[1], L. Lombardi[2], W. Sire[1], J. Simpson[3], G. Butler[2], G. Lee[1]
[1]University College Dublin, School of Chemistry & Chemical Biology, Dublin 4, Ireland
[2]University College Dublin, Conway Institute for Biomolecular & Biomedical Research, Dublin 4, Ireland
[3]University College Dublin, School of Biology & Environmental Science, Dublin 4, Ireland

Backgrounds

Candida albicans is the lead fungal pathogen of nosocomial bloodstream infections in the US and causes 400,000 incidents worldwide annually. Rapid and inexpensive diagnosis and treatment of fungal infections is crucial for prevention of fatalities. Nanoparticles have potential to serve as therapeutics, contrast agents for MRI and a tool for sample preparation.

Objectives

Little is known about how nanoparticles interact with the yeast cell wall, which is crucial to nanoparticle design. We have characterized the interaction of carboxyl-functionalized nanoparticles of various diameters and surface charge with the exterior cell wall of C. albicans hyphae and identified an optimal cell surface target.

Methods

Using laser scanning confocal microscopy and an image analysis protocol, we compared the adsorption of nanoparticles to the hyphal cell wall of mutant strains deficient in expression of certain cell-surface proteins.

Conclusions

A highly significant reduction in particle binding was observed with an Δals3 strain compared to isogenic and clinical isolate strains, suggesting Als3 is the main mediator of carboxyl nanoparticle adhesion. In the absence of Als3, nanoparticles bind to germ tubes in a pattern resembling the localization of Als1. This suggests that Als1 may also play a role in binding. Additionally, we showed that surface charge influences binding – positively charged amine-functionalized nanoparticles fail to bind to the hyphal cell wall unlike negatively charged carboxyl-functionalized nanoparticles. This research suggests Als3 could be a useful target for nanoparticle mediated diagnostics and therapeutics, and provides direction for the optimal size and surface characteristics of nanoparticles for binding to the fungal cell wall.

FEMS7-3174
Biotechnology / Synthetic Biology / Systems Biology - Part II

SELECTION OF AN EFFICIENT PROMOTER AND ITS APPLICATION IN TOYOCAMYCIN PRODUCTION IMPROVEMENT IN STREPTOMYCES DIASTATOCHROMOGENES 1628

Z. Ma[1], *X. Yu*[1]
[1]*China Jiliang University, College of Life Sciences, Hangzhou, China*

Backgrounds

The selection of efficient promoter is usually very crucial for gene expression and metabolic engineering in Streptomycetes.*Streptomyces diastatochromogenes* 1628 was shown to produce the nucleoside antibiotic toyocamycin (TM).However, to our knowledge, promoter engineering in *S. diastatochromogenes* 1628 has not been performed so far.

Objectives

Promoters *kasO*p*,SPL-21 and SPL-57, were shown to be much stronger than *perm*E* in different streptomycetes strains. In this study we investigated whether *kasO*p*, SPL-21 and SPL-57 can be used in *S. diastatochromogenes* 1628.

Methods

Promoter activities were determined by using the β-glucuronidase base assay (GUS-assay).In addition the promoters were used to over-express the gene *toyF* that encodes an adenylosuccinate lyase involved in TM biosynthesis.

Conclusions

Our results indicate that all tested promoters can be used to express genes in *S. diastatochromogenes* 1628. Interesting, promoter SPL-21 showed the strongest transcriptional and expression level and gave rise to a 5.2-fold increase in GUS activity compared with control. In order to improve TM production, the promoters were used to control expression of *toyF*. This gene encodes an adenylosuccinate lyase involved in TM biosynthesis. Among all different recombinant strains, the strain 1628-21F, in which over-expression of *toyF* gene was driven by SPL-21, exhibited the largest increase in TOYF activity and TM production. In a 5-l fermenter this strain produced more than 2 times more TM compared with the wild-type strain.

FEMS7-2578
Biotechnology / Synthetic Biology / Systems Biology - Part II

MICROBIOLOGICAL CONDITION AND ANTIBACTERIAL ACTIVITY OF MEDICINAL SYRUP AND ELIXIRS COMMERCIALIZED IN THE CENTRAL MARKET OF SAO LUIS-MA/BRAZIL

J.R. Sá Melo[1], S.A.M. Alvares Martins[1], S.F. Rocha Constantino[1], W.D. Machado Maciel[1], M. Santos Costa Penha[2], M. Bispo Pinheiro Camara[3], T.D. Val Lima[4], R.D.J. Mendes do Lago Moraes[4], S. Cristina Maia[4], G. Silva Nunes[1], F. Costa Moraes[5]
[1]Faculdade Estácio de São Luís, Biomedicina, São Luís, Brazil
[2]Instituto Florence de Ensino Superior, Farmácia, São Luís, Brazil
[3]IFMA, Química, São Luís, Brazil
[4]Faculdade Estácio de São Luís, Enfermagem, São Luís, Brazil
[5]Faculdade Estácio de São Luís, Enfermagem/Biomedicina, São Luís, Brazil

Backgrounds

In Brazil, the use of medicinal plants to treat various diseases is a widely adopted alternative. Many people resort to home-made preparations such as medicinal syrup and elixirs, medicinal beverages prepared by healers from plants. However, due to the conditions of preparation of these products, there is concern regarding the hygienic and sanitary qualities of it. Nevertheless, researches with medicinal plants are being carried out for the discovery of new antibacterial substances.

Objectives

Evaluate the microbiological quality and the antibacterial activity of medicinal syrup and elixirs commercialized in the central market of São Luís-MA/BRA.

Methods

Were used a honey-based syrup with plants and three root-based elixirs with herbs and leaves, commercialized in the central market of São Luís-MA/BRA. The microbiological parameters were investigated following the Pharmacopoeia methods for the investigation of faecal coliforms, heterotrofic microorganisms and fungi. The antibacterial activity was investigated from the techniques: disk-diffusion, agar drilling and microdilution. Chloramphenicol 0,01mg/mL was used as positive control, and distilled water as negative control. The evaluated microorganisms were: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 14028) e *Pseudomonas aeruginosa* (ATCC 27853). All tests were duplicated.

Conclusions

The fungal growth in all evaluated products, and the presence of faecal coliforms in one of the elixirs don't meet the hygienic and sanitary conditions recommended by the current legislation.
Only the syrup presented an inhibitory effect against the evaluated bacteria, except the sample of *P. aeruginosa.* In contrast, the elixirs had no effect on the species tested.

FEMS7-1257
Biotechnology / Synthetic Biology / Systems Biology - Part II

OXYGEN-LIMITED GROWTH OF SCHEFFERSOMYCES (PICHIA) STIPITIS FOLLOWS EGLI'S DUAL NUTRIENT LIMITED GROWTH PATTERN

S. Maitra[1], *A. Narang*[1]
[1]*Indian Institute of Technology, Biochemical Engineering and Biotechnology, New Delhi, India*

Backgrounds

Due to the dwindling reserves of fossil fuels, there is a need for sustainable energy sources. Production of bioethanol from agricultural residues may provide such an alternative. *Pichia stipitis* is one of the very few strains that ferment both hexoses and pentoses to ethanol without by-products. **A major issue that prevents the industrial use of *Pichia stiptis* is its explicit requirement of microaerophilic conditions for fermentation. A systematic study is needed to understand if such microaerophilic conditions can be maintained robustly.**

Objectives

A) Development of a systematic approach towards understanding the optimum conditions of *Pichia stipitis* for fermentation

B) Development of a mechanical model that predicts the steady state characteristics

Methods

Pichia stipitis (CBS 6054) was studied systematically in a **chemostat** for ethanol yield and productivity. The C: O ratio in the feed was varied at fixed dilution rate and $k_l a$. **Medium was optimized to ensure the limitations were entirely controlled by the C: O ratio.**

Conclusions

Three definite growth regimes (as observed for different C: N concentrations (Egli et al., 1986)) were obtained i.e. C-limited, dual limited and O-limited. Ethanol production starts as the system enters dual limitation regime and continues throughout the O-limited regime, hence **ethanol production in *Pichia stipitis* is a robust process.** Using an approach similar to that in Egli et al., 1986, **a model has been developed** that predicts the steady state characteristics at any dilution rate.

FEMS7-1487
Biotechnology / Synthetic Biology / Systems Biology - Part II

ENZYMATIC SYNTHESIS OF THE DIPEPTIDE TRP-HIS USING L-AMINO ACID ESTERASE

K. Maki[1], T. Tanaka[1], K. Takagi[2], M. Wakayama[1]
[1]Ritsumeikan University, Life science, Kusatsu, Japan
[2]Ritsumeikan University, Applied Chemistry, Kusatsu, Japan

Backgrounds

Trp-His is the only vasoactive dipeptide known to regulate intracellular Ca^{2+}concentration and prevent the onset of atherosclerosis in vivo. A peptide chain that is longer than a tri-peptide is not absorbed through the mucous membrane of epithelium cells of the small intestine. To date, few studies on anti-atherosclerotic small peptides, except for the tetra-peptide Lys–Arg–Glu–Ser, have been reported. It has also been reported that Trp-His works as a Ca^{2+} channel blocker.

Objectives

The aim of this study was to investigate efficient enzymatic synthesis of Trp-His from tryptophan methyl ester and histidine using L-amino acid esterase. This enzymatic synthesis is useful for industrial applications because it does not have complicated steps like chemical synthesis.

Methods

In the first step, soil microorganisms exhibiting L-amino acid esterase activity were screened. The ability of the isolates to synthesize Trp-His was qualitatively evaluated by a colorimetric method and quantitatively analyzed by HPLC.

Conclusions

Eight microorganisms exhibiting Trp-His synthetic activity were isolated and identified by analysis of the 16S ribosomal RNA gene sequences. Among these isolates, *Pseudomonas* sp. KM1 showed the highest activity. Optimal reaction conditions for the production of L-amino acid esterase by *Pseudomonas* sp. KM1 have been examined. When *Pseudomonas* sp. KM1 was cultured in a medium containing D-glucose and yeast extract as carbon and nitrogen sources, the highest Trp-His synthetic activity, 0.0171 μmol/min/mg, was obtained under the following reaction conditions: 50 mM tryptophan methyl ester, 250 mM histidine, and the enzyme at 40°C and pH 9.0 for 60 min.

FEMS7-2672
Biotechnology / Synthetic Biology / Systems Biology - Part II

NEW SYNTHETIC TOOLS, BASED ON RECOMBINEERING AND CRISPR/CAS9, TO UPGRADE GENOME EDITING OF THE ENVIRONMENTAL BACTERIUM PSEUDOMONAS PUTIDA KT2440

T. Aparicio[1], V. de Lorenzo[1], E. Martínez-García[1]
[1]Centro Nacional de Biotecnología CSIC, Systems Biology Program, Madrid, Spain

Backgrounds

The advent of Synthetic Biology has brought about the possibility to massively manipulate bacterial genomes to efficiently program complex genetic devices. Among the commonly used organisms in synthetic biology, the soil and non-pathogenic bacterium *Pseudomonas putida* KT2440 offers a remarkably capacity as a chassis due to its metabolic versatility and high resistance to organic solvents. The engineering of environmental bacteria has been eased with the availability of a broad collection of molecular tools gathered into the SEVA collection. However, genome engineering is still a time-consuming process. Recently, the use of oligos combined with its protection, delivered by the Ssr-recombinase, enabled genome editing (Aparicio T., et al. 2016). While promising, this method show low frequencies of modification making the selection of mutants lacking inconspicuous phenotypes laborious.

Objectives

On this background, we aimed at further developing this procedure by combining it with the use of the CRISPR/Cas9 system as an efficient counter-selection method of non-mutated sequences in *P. putida*.

Methods

To this end, we cloned the Cas9 gene and the CRISPR array into different SEVA plasmids. To survey its functional efficiency we selected the *pyrF* gene as deletion target. Then, we combined the use of the Ssr protein together with Cas9 and transformed cells with an oligo-DNA and the CRISPR plasmid harboring the proper guide.

Conclusions

The combination of both technologies worked with high efficiency for entering a suite of mutations in the chromosome of *P. putida.* These results pave the way for automated and multiplexed editing of the genome of this biotechnologically important bacterium.

FEMS7-1688
Biotechnology / Synthetic Biology / Systems Biology - Part II

HEALTH AND DISEASE IMPRINTED IN THE TIME VARIABILITY OF THE HUMAN MICROBIOME

D. Martínez Martínez[1], J.M. Martí Martínez[1,2], T. Rubio Martínez-Abarca[1], A. Latorre Castillo[1,3,4,5], A. Moya Simarro[1,3,4,5], C. Peña Garay[1,2]
[1]Institute for Integrative Systems Biology I2SysBio, Systems Biology, Paterna, Spain
[2]Corpuscular Physics Insitute IFIC, Cosmology, Paterna, Spain
[3]Cavanilles Institute of Biodiversity and Evolutionary Biology, Evolutionary Genetics, Paterna, Spain
[4]CIBER in Epidemiology and Public Health, Epidemiology and Public Health, Madrid, Spain
[5]FISABIO, Genomics and Health, Valencia, Spain

Backgrounds

Human microbiota plays an important role in keeping the physiological status of the host healthy. Factors such as dietary changes, antibiotic intake, age and disease are often related to higher fluctuations in the temporal microbial composition. This implies that a healthy person will have a stable microbiota, which means that it will be less susceptible to external perturbations.

Objectives

The main objective of this work is to model the time variability of the human gut microbiota for characterizing quantitatively its stability and relate it with the health status of the person.

Methods

We analyzed 16S rRNA and shotgun metagenomic sequencing (SMS) published data from the gut microbiota of 99 individuals monitored over time. We modeled the microbial populations over time with the Taylor's law, and then characterized their stability with the Langevin equation as the basis of our mathematical model.

Conclusions

Temporal fluctuations in the microbial composition revealed significant differences in all the scenarios studied. This research shows that a fluctuation scaling law describes the temporal changes in the gut microbiota. This law enables the temporal variability of the microbial population to be estimated and quantitatively characterizes the path toward disease via a noise-induced phase transition. The estimation of the systemic parameters for follow-up studies may have clinical use and, more generally, may also have applications in other fields where it is important to know whether a given community is stable or not.

FEMS7-1784
Biotechnology / Synthetic Biology / Systems Biology - Part II

SIMULATING THE METABOLIC RESPONSE AT HIGH AND LOW SALINITY IN THE EXTREMOPHILE CHROMOHALOBACTER SALEXIGENS BY USING THE IFA762 GEM METABOLIC MODEL

L. Martínez Martínez[1], *F. Piubeli*[1], *M. Salvador*[2], *M. Argandoña*[1], *J.J. Nieto*[1], *C. Vargas*[1]
[1]*University of Sevilla, Microbiology and Parasitology, Sevilla, Spain*
[2]*University of Surrey, Faculty of Health and Medical Sciences, Guildford, United Kingdom*

Backgrounds

The broad salt-growing *C. salexigens* is a natural producer of ectoines. However, its biotechnology exploitation requires a comprehensive understanding of its physiology and genetics. In order to deepen the understanding of the complex metabolism of *C. salexigens* and its adaptation to osmotic stress, as well as their relationship with the production of ectoines, we have developed a genome-scale metabolic model (GEM) (iFA762) with capacity to simulate the metabolic response at high and low salinity (0.6 M vs 2.5 M NaCl).

Objectives

To get insight in the complex metabolism of *C. salexigens* and its adaptation to osmotic stress to be used in the production of ectoines by GEM simulation.

Methods

Uniform random samples were used to calculate flux distributions probability in the central metabolism of iFA762 model at different salinities. A comparison with experimental results was also carried out.

Conclusions

In this assay of random sampling, fluxes distribution of the most relevant reactions of central metabolism and ectoines synthesis were determined and a salinity-dependent distribution was found for some fluxes. However, a non-significant distribution in some of them were also observed. This agrees with previous experimental results that indicated that an overflow is produced due to the non-adaptation of the TCA. These results showed the capacity of the iFA762 model to simulate metabolic response at different salinities and will help us to develop new metabolic engineering strategies for the optimization of ectoines producer strains.

Funding: MINECO/FEDER(BIO2015-63949-R) y Junta de Andalucía(P11-CV1-7293 MO).

FEMS7-1529
Biotechnology / Synthetic Biology / Systems Biology - Part II

D-LACTIC ACID PRODUCTION WITH METABOLIC ENGINEERED ESCHERICHIA COLI FROM SUGARCANE BAGASSE AND CORN STOVER HYDROLYSATES

A. Martinez[1], *A. Vargas-Tah*[1], *J. Utrilla*[1], *B. Trujillo-Martínez*[1], *M.E. Rodríguez-Alegría*[1], *G. Gosset*[1]
[1]*Instituto de Biotecnología - UNAM, Cellular Engineering and Bioacatalyst, Cuernavaca, Mexico*

Backgrounds

D-lactic acid (D-LA) can be used for a wide array of applications including the production of the biodegradable biopolymer Poly-Lactic-Acid. D-LA production from plant hydrolysates is required for low cost production.

Objectives

To develop *Escherichia coli* strains that preferentially produces D-LA from pentoses and hexoses and lignocellulosic hydrolysates.

Methods

A D-lactogenic strain was obtained using metabolic engineering tools and adaptive evolution. This evolved D-lactogenic *E. coli* strain, named JU15, was sequenced and the following genotype was revealed: *ΔpflB, ΔadhE, ΔfrdA, ΔxylFGH, gatC S184L, ΔmidarpA, Δreg 27.3 kb*, being the galactitol mutant (GatC S184L) responsible for efficient xylose transport. The L-lactic and acetic acid production pathways were deleted in JU15, resulting strain AV03 (JU15 Δ*poxB*, Δ*ackA-pta,* Δ*mgsA*).

Conclusions

Results showed that strain JU15 produces D-LA with high yield and productivity in laboratory simulated hydrolysate media and actual sugar cane bagasse hemicellulosic hydrolysate with a minimal nutrient addition in pH controlled batch fermenters. Strain JU15 showed sequential carbon source utilization and acetic acid production. The AV03 strain showed simultaneous consumption of glucose and xylose and no acetic acid production in laboratory simulated hydrolysate and sugar mixtures (pentoses and hexoses) from corn stover hydrolysates. The D-LA yield from hydrolysate sugars was close to 0.95 g/g_{sugars} in all cases and volumetric productivities above 1 g/L h were obtained in batch cultivations from 0.2 to 10-liter fermenter scale.

FEMS7-1533
Biotechnology / Synthetic Biology / Systems Biology - Part II

METABOLIC ENGINEERING OF ESCHERICHIA COLI FOR R-3-HYDROXYBUTYRATE PRODUCTION WITH INCREASED AVAILABILITY OF NADPH

A. Martinez[1], M. García-Benítez[1], L.M. Martínez-Mejía[1], G. Hernández-Chávez[1], G. Gosset[1]
[1]Instituto de Biotecnología - UNAM, Cellular Engineering and Bioacatalyst, Cuernavaca, Mexico

Backgrounds

R-3-hydroxybutyryl-CoA is the biochemical precursor used by several microorganisms to produce polyhydroxybutyrate, a biodegradable bioplastic. Moreover, R-3-hydroxybutyryl-CoA can be enzymatically converted to R-3-hydroxybutyrate (R-3-HB), which is a chemical building block used in the manufacture of antibiotics, biofuels, pharmaceuticals and in the controlled synthesis of several polyhydroxyalkanoates with specific composition and length.

Objectives

In this study, the Gram-positive bacterium *Escherichia coli* was metabolically engineered to produce R-3-HB from glucose in mineral media.

Methods

First, to reduce the formation of fermentation co-products, all the genes encoding carbon competing pathways in the phosphoenolpyruvate, pyruvate and acetyl-CoA nodes were eliminated into the chromosome of *E. coli*. Second, as the biochemical conversion of acetyl-CoA into acetoacetyl-CoA and then into R-3-hydroxybutyryl-CoA requires a reduction step that uses NADPH, a gene encoding a glyceraldehyde-3-P dehydrogenase from *Streptococcus mutans*, that specifically uses NADP, was integrated into the chromosome to promote the formation of NADPH when glyceraldehyde-3-P is metabolized to 1,3-bi-phosphoglycerate. Third, the genes encoding the beta-ceto thiolase and the acetoacetyl-CoA reductase from *Azotobacter vinelandii* were cloned in tandem with the homologous thioesterase of *E coli* in an IPTG inducible vector to promote the conversion of acetyl-CoA into R-3-hydroxybutyrate, and the non-fermentative strain was transformed with this plasmid.

Conclusions

Strain characterization in shake flasks and aerated bioreactors, allowed to produce R-3-HBT with a 59% yield of the maximum theoretical, reaching 9.9 g/L in batch cultures when mineral media and glucose were used. However, unexpected production of high amounts of pyruvate and acetate points to further genetic modifications and to use fed-batch cultivation.

FEMS7-1142
Biotechnology / Synthetic Biology / Systems Biology - Part II

BIOTECHNOLOGICAL APPROACHES FOR OBTAINING OF NEW COMPLEX BIOPREPARATION FOR ORGANIC AGRICULTURE

L. Melkonyan[1], G. Avetisova[1], A. Chakhalyan[1], S. Keleshyan[1], Z. Karapetyan[1], S. Stepanyan[1], A. Toplaghaltsyan[1], A. Saghyan[2]
[1]SPC "Armbiotechnology" of NAS of Armenia, Laboratory of Selection of strain-producers of BAS, Yerevan, Armenia
[2]SPC "Armbiotechnology" of NAS of Armenia, Director, Yerevan, Armenia

Backgrounds

The world community comes across with the problem of increasing exploitation of mineral fertilizers. Mineral fertilizers and many toxic chemicals damage the biosphere. They also contribute to various human diseases by affecting fruits and vegetables that grow in this contaminated soil. In this aspect to remediate soil and enhance its fertility, the minimisation of exploiting rates of ecologically irrational chemicals and their substitution by ecologically safe fertilizers is of crucial importance. One of the ways to solve this problem is the development and manufacturing of biofertilizer.

Objectives

The aim of study was to develop a technology of complex, ecologically safe biofertilizer.

Methods

Strains of different microorganisms with high nitrogen-fixing activities were isolated from various soils of Armenia by methods described previously *(Yegorov N.S., 1995; Zheldakova R.A., 2003)*.

Conclusions

A selected strain allowed to develop “Ecobiofeed+” biopreparation also consisting of natural minerals and raw plant materials. This complex can be used for versatile plant feedings including nitrogen nutrition and all types of soil fertilization. “Ecobiofeed+” biopreparation increased germination, root striking of seedlings and planting stocks, crops of vegetables and melons, industrial, fodder, fruit and berry cultures, plant immunity, environmental purity and quality of vegetables and fruits.The advantages of this biopreparation are its high efficiency (not washed out from the soil), duration of its effect, early ripening of crops, decrease of some plant diseases, reduction of nitrates in agricultural products and soil regeneration.

FEMS7-2862
Biotechnology / Synthetic Biology / Systems Biology - Part II

BGL-3 FROM TALAROMYCES AMESTOLKIAE: A FUNGAL BETA-GLUCOSIDASE PRODUCED UNDER CARBON SOURCE STARVATION, USEFUL FOR 2G AND 3G BIOFUELS PRODUCTION

J.A. Méndez-Líter[1], *L.I. De Eugenio*[1], *M.J. Martínez*[1]
[1]*Centro de Investigaciones Biológicas CIB-CSIC, Environmental Biology, Madrid, Spain*

Backgrounds

Plant biomass represents one of the most abundant and underutilized biological resources, and is a promising source of material for 2G biofuels production. Recently, 3G biofuels, based in algae biomass, have emerged as an alternative and the discovery of enzymes applicable for 2G and 3G processes, represent an interesting alternative for industry.

Objectives

In this work, we describe the production, purification and characterization of a β-glucosidase from *T. amestolkiae* (BGL-3), and its effectiveness in hydrolysis and saccharification of several substrates.

Methods

BGL-3 was produced in Mandels medium induced with several carbon sources, analyzing the expression of the *bgl3* gene by qRT-PCR. The saccharification of wheat straw was carried out with Celluclast 1.5L (Novozymes), as commercial source of cellulases, with or without BGL-3 or N-50010 (Novozymes), as source of β-glucosidase activity, to complement Celluclast 1.5L.

Conclusions

BGL-3 was produced with every inductor tested, suggesting that its production was carbon-source independent. Analysis of *bgl3* expression levels showed that its transcription is triggered under carbon starvation. Purified BGL-3 showed good thermal stability and high efficiency over *p*NPG and cellooligosaccharides. In wheat straw saccharification, BGL-3 worked better than the commercial cocktail N-50010 supplementing Celluclast 1.5L. Besides, its versatility was remarkable, since BGL-3 hydrolyzed the algal polysaccharide laminarin (1,3-β bonded), with higher yields than a commercial laminarinase. It is the first time that such versatility is described in a 1,4-β-glucosidase, which makes BGL-3 outstanding for a potential application for production of 2G and 3G biofuels.

Thanks to MINECO BIO2015-68387-R and CM S2013/MAE-2907 for funding.

FEMS7-1176
Biotechnology / Synthetic Biology / Systems Biology - Part II

BIOLOGICAL CONVERSION OF WASTE LIGNOCELLULOSIC BIOMASS FOR ALCOHOL PRODUCTION

L. Mezule[1], B. Dalecka[1], T. Juhna[1]
[1]Riga Technical University, Water Research Laboratory, Riga, Latvia

Backgrounds

Conversion of lignocellulosic biomass, e.g., grass, agricultural and wood processing waste, into valuable chemical products, e.g., biofuel, is closely linked with the selection of the most efficient pre-treatment and hydrolysis technique to produce fermentable sugars. Biological hydrolysis is regarded as one of the potential approaches over to classically applied chemical treatments, however, it is often linked with high costs of enzymes and long conversion time. At the same time it has been acknowledged that fermentation feed produced via biological hydrolysis has less fermentation inhibitors and is more suitable for alcohol producing microorganisms.

Objectives

In this research laboratory made enzyme products from white rot fungi were tested in a simple pre-treatment/hydrolysis assay to produce fermentable sugars. The suitability of these preparations for yeast and *Clostridium* fermentation processes were further tested in batch and pilot scale.

Methods

The results demonstrated that it is possible to produce fermentable sugar of up to 80% from the theoretical yield from lignocellulosic biomass within 24 hours of hydrolysis. The obtained sugar solutions were applicable for alcohol production and did not showed any significant effect ($p > 0.05$) on microorganism growth properties.

Conclusions

It was possible to design a simple, efficient biological biomass conversion technology to produce alcohols.

Acknowledgement: This work has been supported by Latvian National Research Programme No. 2014.10-4/VPP-1/27 “LATENERGI”.

FEMS7-0478
Biotechnology / Synthetic Biology / Systems Biology - Part II

BREVIBACILLUS LATEROSPORUS STRAIN BGSS7: ISOLATE FROM SILAGE WITH A BROAD SPECTRUM OF ANTIMICROBIAL ACTIVITY AGAINST GRAM POSITIVE AND GRAM NEGATIVE MULTIRESISTANT PATHOGENS

S. Jovanovic[1], N. Mirkovic[1], M. Miljkovic[1], M. Malesevic[1], J. Lozo[2], B. Jovcic[2], G. Vukotic[2], B. Filipic[3], K. Novovic[1], M. Kojic[1]
[1]Institute of Molecular Genetics and Genetic Engineering- University of Belgrade, Laboratory for Molecular Microbiology, Belgrade, Serbia
[2]Faculty of Biology- University of Belgrade, Biochemistry and Molecular Biology, Belgrade, Serbia
[3]Faculty of Pharmacy- University of Belgrade, Department of Microbiology and Immunology, Belgrade, Serbia

Backgrounds

Antimicrobial peptides are short-sequence peptides with broad spectrum activity against bacteria, fungi and viruses. They are divided into two groups based on biosynthesis: i) non-ribosomally synthesized peptides, which are mostly produced by bacteria and ii) ribosomally synthesized peptides. *Brevibacillus laterosporus,* previously classified as *Bacillus laterosporus*, can produce antimicrobial peptides, such as lolatin A and lactolisperin.

Objectives

The aims of present study was to (i) isolate bacteria from silage, (ii) screen for production of antimicrobial peptides and (iii) identify of bacterial strains with antimicrobial activity against various pathogens.

Methods

For isolation of bacteria, 10 g of silage was re-suspended in 90 mL of peptone water, submitted to serial tenfold dilution and spread on the surface of LB, GM17 and MRS agar plates. After incubation at 37°C for 48h, fifty colonies per plates were randomly selected and transferred in corresponding broth and incubated o/n at 37°C. Agar well diffusion test was used for detection of antimicrobial activity.

Conclusions

Among randomly selected isolates from silage, only one BGSS7 showed strong inhibitory activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which was identified as *Brevibacillus laterosporus* by 16S rRNA gene sequencing. This is the first report of isolation of *B. laterosporus* from the silage with broad antagonistic potential. In this study, we gave preliminary data about antimicrobial activity of BGSS7 against Gram positive and negative pathogens; however localization of the gene(s) coding for antimicrobial peptide production, as well as determination of structure of the antimicrobial peptide is still in progress.

FEMS7-0489
Biotechnology / Synthetic Biology / Systems Biology - Part II

ANTIFUNGAL ACTIVITY OF VOLATILE ORGANIC COMPOUNDS FROM ENDOPHYTIC GEOTRICHUM CANDIDUM AGAINST RICE PATHOGEN RHIZOCTONIA SOLANI

A. Mookherjee[1], *M. Maiti*[1]
[1]*Indian Institute of Technology- Kharagpur- India, Biotechnology, Kharagpur, India*

Backgrounds

Antimicrobial volatile organic compounds (VOCs) from biological sources have enormous potential as a replacement of chemical fumigants. For the past few decades, scientists were able to discover different microbes with the ability to synthesize antimicrobial VOCs. Endophytic microbes, one of the prospective source, have the capacity to produce vast numbers of bioactive agents due to their presence in a specialized niche.

Objectives

The objective of this study was to detect antimicrobial activity, if any, in a laboratory isolated endophytic fungi with fruity fragrance. Based on the qualitative profile of its VOCs, metabolic engineering experiments were set up for enhancement of its antimicrobial activity.

Methods

The endophytic fungi was identified through phenotypic and molecular characterization. Metabolite profile of the fungal VOCs from GC-MS analysis, facilitated to find out prospective targets of precursor feeding for enhancement of its antimicrobial activity.

Conclusions

The endophytic fungi was successfully identified as *Geotrichum candidum* PF005. *G. candidum* PF005 had significant antifungal activity against rice pathogen *Rhizoctonia solani*. The source of carbon as nutrient was identified to be important factor for the production of antifungal VOCs. Presence of esters, hydrocarbons and alcohols were identified in significant proportion from the volatile fraction of *G. candidum* PF005. Antifungal activity against phytopathogenic *R. solani* got improved by feeding the fungal strain PF005 with precursors of 2-phenylethanol and 3-methyl-1-butanol synthesis pathways. Fruity fragrance and qualified presumption of safety status of *G. candidus* make it much more desirable candidate to utilize it as mycofumigant to control phytopathogen *R. solani* of crop plants.

FEMS7-1003
Biotechnology / Synthetic Biology / Systems Biology - Part II

ASSESSING THE ROLE OF CANDIDATE TRANSPORTERS IN THE METABOLIC COMPLEMENTATION BETWEEN BLATTELLA GERMANICA AND ITS ENDOSYMBIONT BLATTABACTERIUM

M. Muñoz Benavent[1], C. Picazo[2], R. Domínguez-Santos[1], J. Peretó[1], C. García-Ferris[1], A. Latorre[1]
[1]Institute for Integrative Systems Biology I2SysBio, University of Valencia, Paterna, Spain
[2]Institute for Integrative Systems Biology I2SysBio, Univeristy of Valencia, Paterna, Spain

Backgrounds

Symbiosis is a key biological interaction in evolution processes. It is present all over the biological kingdoms and is especially important in insects. It is the case of the german cockroach (*Blattella germanica*), which has an endosymbiont: *Blattabacterium cuenoti*. This is a flavobacterium who lives inside specialised cells called bacteriocytes, located in the fat body of the cockroach. The biological interaction between both organisms has been studied and it has been hypothesised that it is due to a metabolic complementation based on the recycling of nitrogen. The bacterium would complete the uricolityc pathway and produce ammonia from urea, which must be transported into *Blattabacterium*. The ammonium would be organically fixed by the host in the form of glutamine that would be transported into *Blattabacterium* to be used in the biosynthesis of essential amino acids.

Objectives

The objective of this study is to identify some of the molecular elements involved in this complementation, in particular the transporters that allow the flow of molecules through the membranes that separate the cytoplasm of both organisms (*Blattabacterium* / *Blattella* interphase).

Methods

For this we have analyzed the genome of *Blattabacterium* in search of candidate proteins (alt. transporters) in order to express them in yeast and carry out functional complementation tests. These experiments consist of cloning our candidate proteins in yeast mutants for the transport of the molecules involved in the hypothetical metabolic complementation.

Conclusions

Later they will be cultivated in restrictive media that will allow to evaluate their function.

FEMS7-2615
Biotechnology / Synthetic Biology / Systems Biology - Part II

SCREENING AND CHARACTERIZATION OF INDIGENOUS MICROALGAL STRAINS FOR PRODUCTION OF BIOMASS AND CAROTENOIDS UNDER PHOTOAUTOTROPHIC AND MIXOTROPHIC GROWTH CONDITIONS

T. Mutanda[1], *N. Zibula*[1]
[1]*University of KwaZulu-Natal, Microbiology- School of Life Sciences, Durban, South Africa*

Backgrounds

Microalgae are currently gaining considerable attention for biofuels and biochemicals production by the biorefinery approach. Carotenoids are accessory pigments interacting with harmful reactive oxygen species (ROS) thereby acting as free radical quenchers, singlet oxygen scavengers and lipid antioxidants and are reportedly beneficial to human well-being.

Objectives

To screen and characterize microalgae producing carotenoids.

Methods

In this study, 20 unique wild-type indigenous microalgal strains were isolated from diverse aquatic habitats in KwaZulu-Natal Province, South Africa. The axenic microalgal strains were propagated in modified seawater and BG-11 growth media for 15 days in closed bioreactors and screened for carotenoid production by non-destructive chromatographic and spectrophotometric analysis. Two microalgal stains (strain 1 and strain 2) showed potential for β-carotene production and were subsequently subjected to photoautotrophic and mixotrophic growth conditions. Both strains showed robust growth and biomass accumulation under photoautrophic growth conditions. Total β-carotene production was 0.325 and 0.311 mg/mL for strain 1 and strain 2 respectively after 15 days of uninterrupted growth under photoautotrophic growth conditions. Several carbon sources were investigated and strain 1 and strain 2 grew maximally in media supplemented with glycerol and sodium acetate respectively. Microalgal strain 1 and strain 2 achieved highest total β-carotene concentration of 0.372 mg/mL and 0.385 mg/mL respectively, in media supplemented with sodium acetate after 15 days of growth.

Conclusions

Therefore, mixotrophic growth conditions enhanced β-carotene production by the 2 strains as compared to photoautotrophy. This study demonstrated suitable growth conditions for production of biomass and carotenoids by two indigenous microalgal strains with potential for biotechnological applications.

FEMS7-3234
Biotechnology / Synthetic Biology / Systems Biology - Part II

CHARACTERIZATION OF UNEXPLORED QATARI BACILLUS THURINGIENSIS STRAINS FOR IDENTIFYING POTENTIAL BIO-INSECTICIDE PRODUCERS TARGETING DIPTERAN INSECTS

S. Jaoua[1], K. Nair[1]
[1]Qatar University, Department of Biological & Environmental Sciences, Doha, Qatar

Backgrounds

Many fatal human diseases are contributed to the *Dipteran* vectors like mosquitoes. The measures of controlling them are mostly chemical based. Use of these chemical insecticides are considered one of the crucial environmental problems today. One of the sustainable and biological alternative is the use of larvicidal endotoxin proteins produced by the gram-positive soil bacterium, *Bacillus thuringiensis* (*Bt*) in the form of spherical crystals.

Objectives

Our objective is to screen the unexplored Qatari microbial communities to identify *Bacillus thuringiensis* strains that would be a good candidate to produce bio-insecticide.

Methods

We have isolated 700 strains and have found 441 isolates that produce spherical crystals. After intrinsically studying the spherical crystals by Scanning electron microscopy, we have categorized the 441 isolates based on their protein and plasmid patterns. Among these, we have 19 isolates that have molecular and biochemical characteristics indicative of insecticidal activity. We have also evidenced structural instability in the plasmid of these isolates when evaluating the endotoxin genes by PCR (polymerase chain reaction) amplifications.

Conclusions

In conclusion, considering the extreme climatic conditions of Qatar, the microbial community offers unique *Bt* strains and our analyses show that they have the potential to replace the chemical insecticides.

FEMS7-2799
Biotechnology / Synthetic Biology / Systems Biology - Part III

EVOLUTIONARY HISTORY OF FUNGAL VERSATILE-LIPASES FROM THE ORDER AGARICALES

J. Barriuso[1], *M.J. Martínez*[1]
[1]*Centro de Investigaciones Biológicas CIB-CSIC, Environmental Biology, Madrid, Spain*

Backgrounds

Lipases (EC 3.1.1.3) and sterol esterases (EC 3.1.1.13) are thoroughly used in industrial applications. Among fungi some extracellular proteins combine properties of both enzymes, and there are known as “Versatile carboxylic ester hydrolases” (abH03.01). Agaricales is the largest clade of mushroom-forming fungi and one of the most ancient orders, comprising wood saprophytes with biotechnological interest. On the other hand, the *in silico* mining of fungal genomes and metagenomes has been shown as an alternative for searching novel lipases, while exploring the evolutionary history and resurrecting intermediate ancestral forms of enzymes can help to explain the mechanistic basis of enzymes function and disclose new functionalities.

Objectives

In this work we explored the presence of genes encoding putative “Versatile lipases” from the order Agaricales. We reconstructed the molecular evolution of these enzymes and inferred the sequence of their ancestral intermediate forms. The potential properties of the candidates are discussed on the basis of their three-dimensional (3D) model structure, the presence and hydrophobicity of the lid, and the substrate binding tunnel.

Methods

We used conserved motifs, sequence and phylogenetic analyses, and three-dimensional modeling to look for candidate genes in public fungal genomes. Moreover, we reconstructed the molecular evolution of these enzymes using novel phylogenetic approaches (PAML4.8).

Conclusions

The evolutionary history of the putative lipases revealed an increase on the length and hydrophobicity of the lid region, as well as in the size of the substrate binding pocket, during evolution time. These facts suggest the enzymes’ specialization towards certain substrates and their subsequent loss of promiscuity.

FEMS7-0277
Biotechnology / Synthetic Biology / Systems Biology - Part III

LESS IS MORE: HYDROLYSIS OF POLYESTERS IS ENHANCED BY A TRUNCATION OF AN ESTERASE

A. Biundo[1], R. Doris[2,3], G. Steinkellner[4], K. Gruber[5]
[1]Polymer and Environmental Biotechnology, Austrian Centre of Industrial Biotechnology, Tulln, Austria
[2]Institute for Environmental Biotechnology, Department of Agrobiotechnology- IFA-Tulln, Tulln, Austria
[3]Polymers and Environmental Biotechnology, Austrian Centre for Industrial Biotechnology, Tulln, Austria
[4]Austrian Centre of Industrial Biotechnology, Austrian Centre of Industrial Biotechnology, Graz, Austria
[5]Institute of Molecular Bioscience, University of Graz, Graz, Austria

Backgrounds

Polymers frequently used for one-way applications like packaging are preferably biodegradable, albeit non-biodegradable polyesters are mostly used. Biodegradability of the aliphatic/aromatic copolyester poly(butylene adipate-*co*-terephthalate) (PBAT) has been investigated, showing biological decomposability under composting conditions. However, little is known about its anaerobic hydrolysis while large amount of food packaging ends up in biogas plants. The enzyme EstA from *Clostridium botulinum* (Cbotu_EstA) actively hydrolyzed PBAT while it failed to act on polyethylene terephthalate (PET). Yet, enzymes would allow mild decomposition of widely used PET enabling recycling of the monomeric building blocks.

Objectives

The enhancement of the hydrolase activity with regard to polyester hydrolysis was carried out by fusion of hydrophobic domains, improving the biocatalyst adsorption on the hydrophobic polymer surface, or by substitution of specific residues, enlarging the active site of the enzyme. The deletion of the Cbotu_EstA N-terminal domain can satisfactory combine both approaches.

Methods

The 3D structure analysis of Cbotu_EstA revealed the presence of an N-terminal domain covering the lid structure and a hydrophobic patch. Chemoluminescence and HPLC analysis determined the adsorption onto hydrophobic surfaces and the hydrolyzing activity of the enzyme, respectively. Furthermore, analysis of the kinetic constants made the catalytic activity investigation of the two forms of the enzyme possible.

Conclusions

Surface engineering successfully produced a highly active Cbotu_EstA variant which was able to hydrolyze PET. Truncation of the N-terminal domain of Cbotu_EstA improved the adsorption of the enzyme on hydrophobic polyester surfaces and enhanced their hydrolysis eight times more compared to the wild-type enzyme, based on released monomers quantification.

FEMS7-1927
Biotechnology / Synthetic Biology / Systems Biology - Part III

COMPARATIVE GENOMIC ANALYSIS OF NOVEL ACINETOBACTER SYMBIONTS: A COMBINED SYSTEMS BIOLOGY AND GENOMICS APPROACH

V. Gupta[1], *U. Sood*[1], *Y. Singh*[1], *R. Lal*[2]
[1]*University Of Delhi, Lab 112- Department Of Zoology, Delhi, India*
[2]*University Of Delhi, Lab 115- Department Of Zoology, Delhi, India*

Backgrounds

BACKGROUND : The increasing trend of antibiotic resistance in *Acinetobacter* is a matter of global concern as it drastically limits the range of therapeutic agents required to treat multidrug resistant (MDR) infections. To concur these pathogenic bacteria, we planned a network theory based study to identify the regulatory proteins.

Objectives

OBJECTIVES: Comparative genomics analysis was performed in between 3 strains of *Acinetobacter* spp. isolated from sheep and one from the insect gut. Sequencing and annotations were done to gain the details in pathogenicity of *Acinetobacter* spp.

Methods

METHODS: In this study we have applied multi-omics approach to explore system ecology of *Acinetobacter* spp. using genomics and systems biology. Four novel strains of *Acinetobacter* spp. (pathogenic and non-pathogenic) were phylogenetically classified and further used to computationally construct subnetwork/modules using the network theory methods

Conclusions

RESULTS: We identified eight major key regulatory genes (hubs), guaA, rpsB, rpsI, rpsL, rpsE, rpsC, rplM and trmD , which have functional roles demonstrating a hierarchical scale-free fractal protein-protein interaction network. Two unique key hubs, *i.e*., guaA and guaB played an important role in *Acinetobacter* sp. isolated from insect gut. The hub guaA was comparatively more important than guaB as it help in effective module regulation. The rpsI gene was important in all the studied novel strains of *Acinetobacter* spp. The rplM gene was specific only to strains isolated from sheep gut.

CONCLUSIONS: We found that three genes hub, rpsM, rpsB and rpsI, were involved in the regulation of overall network topology across all *Acinetobacter* strains in this study. Combining the multi-omics approach have revealed the system ecology of *Acinetobacter* ecotypes for further investigation of these hubs if they are effective drug target for *Acinetobacter* spp. infections.

FEMS7-3278
Biotechnology / Synthetic Biology / Systems Biology - Part III

CARBOXYLATE PLATFORM CHEMICALS AND BIOFUELS FROM BIOWASTE

E. Hakalehto[1,2], S. Schwede[3], R. Laatikainen[4], E. den Boer[5], L. Piiroinen[1]
[1]Finnoflag Oy, P.O. Box 262, 70101 Kuopio, Finland
[2]Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland
[3]School of Business, Society and Engineering, Mälardalen University, Västerås, Sweden
[4]School of Pharmacy, University of Eastern Finland, Kuopio, Finland
[5]Wrocław University of Science and Technology,
Department of Environmental Protection Engineering, Wroclaw, Poland

Backgrounds

A significant share of the fuels and chemicals used in the economy could be replaced by substances produced from biomass sources. Recent international and European development strategies attribute high priority to the growth of bioeconomy sector. A radical change of production, consumption, processing, storage, recycling and disposal of biological resources is needed, in order to cope with an increasing global population, rapid depletion of many resources, increasing environmental pressures and climate change.

Objectives

Micro-organisms provide tools for circulating the substances in the industrial ecosystems. This paper focuses on the production of carboxylic platform chemicals from industrial and municipal kitchen biowaste using non-aceptic conditions. Verification of this technology has been done based on pilot tests in 3 regions – in Finland, Poland and Sweden.

Methods

The biorefinery technology was implemented in a pilot scale with effective reactor volumes of 200-300 L under anaerobic and microaerobic conditions. The production organisms belonged to the genera *Clostridium* and *Klebsiella*. Selected biowaste streams were subjected to enzymatic pretreatment prior to the inoculation.

Conclusions

The results showed high conversion rates achieved from mixed substrates. The dominating end products were volatile fatty acids (VFAs), ethanol and CO_2. Lower levels of other gases, such as H_2, CH_4 and H_2S, were formed. The conversion rates of glucose to the main fermentation products reached up to 0.81 mol/mol. Valeric acid was one of the main VFA products from the potato waste carbohydrates, whereas in trials on chicken litter, the valerate derived mostly from the amino acids of the biowaste proteins.

FEMS7-1219
Biotechnology / Synthetic Biology / Systems Biology - Part III

OLEOGELS ELABORATION USING LIGNIN SOLUBILIZED BY STREPTOMYCES FROM AGRICULTURAL RESIDUES

A. Blánquez[1], J. Rodríguez[2], F. Guillén[2], S. Morales[2], C. Valencia[3], J.M. Franco[4], M.E. Arias[2], M. Hernández[2]
[1]University of Acalá, Dpto. Biomedicine and Biotechnology, Alcalá de Henares, Spain
[2]University of Acalá, Department of Biomedicine and Biotechnology, Alcalá de Henares, Spain
[3]University of Huelva, Department of Chemical engineering-
Physical Chemistry and Materials Science, Huelva, Spain
[4]University of Huelva,
Department of Chemical engineering- Physical Chemistry and Materials Science, Huelva, Spain

Backgrounds

Lignin is an aromatic biopolymer traditionally considered a waste product in industrial processes and agricultural practices causing serious pollution problems. At present, lignin is considered a renewable biopolymer for biotechnological applications. The suitability of *Streptomyces* to solubilize lignin from grasses residues, instead of to mineralize this polymer, could be a good strategy for biotechnological purposes.

Objectives

The aim of this work is to set up the optimal conditions to solubilize lignin from agricultural residues using selected *Streptomyces* strains to be applied for oleogels elaboration.

Methods

Different *Streptomyces* strains were cultured for 7 days under Solid-State Fermentation conditions (SSF) on agricultural residues. Transformed substrates were extracted with water or 0.1 M NaOH and extracts were acidified with HCl. Finally, water or alkali-extracted lignin were gravimetrically estimated. Moreover, xylanase, mannanase and laccase activities were determined along the time course of growth. Selected strains were also cultured under SSF conditions supplemented with radicals promoting agents such as quinones and Fe^{3+}-EDTA. Prior to this, the Fe^{3+} content of substrates (60 ± 3 µg g^{-1}) were determined by Atomic Absorption Spectrophotometry. For oleogels preparation alkali-lignin was modified with 1,6-hexamethylene diisocyanate.

Conclusions

An increase in 10-20% of alkali-lignin yield was achieved with selected strains compared to that obtained from uninoculated substrates.

Under radical producing conditions, the colonization ability of strains was not affected being the yield of water-extracted lignin 20 % higher than that obtained in non-supplemented cultures with radical production promoters.

Preliminary assays carried out with oleogels elaborated with alkali-lignin showed suitable thermal resistance and rheological characteristics.

FEMS7-3175
Biotechnology / Synthetic Biology / Systems Biology - Part III

CONSTRUCTION AND CHARACTERIZATION OF MICROBIAL CONSORTIA FOR KERATIN DEGRADATION

D. Kang[1], S. Jacquiod[2], J. Herschend[1], S.J. Sørensen[1]
[1]University of Copenhagen, Department of Biology, Copenhagen, Denmark
[2]INRA, UMR 1347 Agroécologie, Dijon, France

Backgrounds

Europe, especially Denmark, heavily relies on import of soy protein for feed in its animal production. Keratins refer to a group of insoluble, tough and recalcitrant protein material generated in large quantities as a waste-product in commercial slaughterhouses, originating from bristles and hooves. Utilization of microbial consortia can play a crucial role as natural biodegradation process for keratin. It can be transformed into more valuable feedstock through the release of small peptides and amino acids by microbial degradation.

Objectives

Identify microbial communities for keratin degradation and construction of stable microbial consortia for industrial applications.

Methods

A soil-born microbial consortium was enriched on keratin as the sole carbon source in sequential batch cultivations at room temperature and characterized by application of next generation sequencing and specific keratin degradation assays.

Conclusions

During six enrichment cycles, the procedure selected for stable and efficient keratin degrading microbial consortia, mainly constituted by members of bacteroidetes and proteobacteria phyla. For the sake of industrial applications, we used a dilution-based method to reduce the diversity and isolate key component strains involved in efficient keratin degradation, while excluding potential cheaters and ease of controllable outputs. The consortia were structurally stable with the co-existence of four major microbes, comprising aerobic bacterial genera *Chryseobacterium*, *Stenotrophomonas*, *Pseudochrobactrum, Acinetobacter*. Residual substrates proportion is similar when comparing with using the microbial consortium without dilution and approximate 20% after five days. This work has potential applications for a vast range of areas including food and feed, fisheries, biotechnologies and agriculture.

FEMS7-2641
Biotechnology / Synthetic Biology / Systems Biology - Part III

MARINE BACTERIAL COMMUNITY FUNCTIONAL METAGENOMICS AND GENETIC ANALYSIS TO ISOLATE AND CHARACTERISE NOVEL BIOACTIVE COMPOUNDS TOXIC TOWARDS THE NEMATODE CAENORHABDITIS ELEGANS

J. Nappi[1], *F. Ballestriero*[1], *C. Campi*[1], *S. Egan*[1]
[1]*University of New South Wales, School of Biotechnology and Biomolecular Science, Sydney, Australia*

Backgrounds

Due to the rise of microbial resistance to antibiotics, there is an urgent need for the discovery and development of new antimicrobial drugs. Marine environments and in particular host-associated bacteria are proving to be a promising source of novel bioactive metabolites. Moreover modern –omics tools enable researchers to tap into the vast diversity and potential of uncultured microorganisms.

Objectives

The aim of this study was to use a culture independent functional metagenomic approach to identify and characterise new bioactive metabolites isolated from bacterial epiphytes of the seaweed *Ulva australis*.

Methods

We screened an *E. coli metagenomic fosmid* library of the microbial community associated with *U. australis* for activity against the model nematode *Caenorhabditis elegans*. Active clones were further analysed with transposon mutagenesis. The resulting mutant clone library was screened for loss of activity and the mutants sequenced to identify the gene clusters involved in the toxicity towards *C. elegans*.

Conclusions

Our library screening resulted in 150 potential active clones and of those, six were confirmed as being toxic to the nematode. One clone (JJ117) was particularly toxic to *C. elegans* and further analysis revealed that it contains DNA sequence with high identity to an unknown marine alpha-proteobacteria (order *Rhodobacterales)*. Bacterial species within this taxa have previously been shown to be associated with the production of antibacterial compounds. Genetic analysis indicates that this clone produces a novel compound involving the activity of one ATP grasp superfamily protein and an Alpha E superfamily protein. Further studies will involve genetic complementation and chemical analysis of JJ117.

FEMS7-0756
Biotechnology / Synthetic Biology / Systems Biology - Part III

IDENTIFICATION, ACTIVITY AND MOLECULAR SCREENING OF PECTINASE ENZYME IN HALOPHILIC BACTERIA FROM SALT LAKE, IRAN

Z. nasrollahzadeh[1], *E. Salehghamari*[1], *M.A. Amoozegar*[1]
[1]*Science, Biology, Tehran, Iran*

Backgrounds

Pectinase is enzymatic complex including pectin methyl esterase, pectin lyase and polygalactoronase. Finding the microorganisms with ability of synthesis of these enzymes in extreme condition such as different salt concentration could have beneficial application in industry.

Objectives

In this research we screened halophilic bacteria from salt lake of Iran and activity and the genes of coding pectinase were studied.

Methods

Isolated bacteria from Urmia, Inche Boron and Gomishan lakes were cultured on selective medium containing pectin as sole carbon source. The positive isolates were identified by I2/kI qualitative test. The activity of pectin esterase, pectin lyase and polygalactoronase enzymes was measured in positive isolates by spectrophotometry at 620, 235 and 595 nm respectively. The pectate lyase gene was amplified using appropriate degenerated primers, in order to screen the gene. Then the products were cloned and sequenced.

Conclusions

Among the 130 studied strains, 17 positive strains were identified for these enzymes of which 10, 6 and 1 belonged to the Gomishan, Inche Boron and Urmia lakes respectively. The results of spectrophotometer showed that most of the produced pectin esterase, pectin lyase and polygalactoronase enzymes were related to isolated strains from the Inche Boron Lake. Although, R_2S_{25} bacterial strain isolated from Inche Boron Lake showed the highest production of these enzymes.
After sequencing the PCR products from 17 positive samples of halophilic bacteria, the phylogenic tree of pectate lyase gene was established to introduce the evolutionary relationship in different strains. In conclusion, the evolutionary algorithm of pectinase gene was different from *16s rDNA* phylogenetic tree.

FEMS7-0437
Biotechnology / Synthetic Biology / Systems Biology - Part III

CONVERSION OF LIGNOCELLULOSE TO JET FUELS WITH MEMBRANE CONCENTRATION

A. Netrusov[1], V. Teplyakov[2], M. Tsodikov[3]
[1]Lomonosov Moscow State University, Microbiology, Moscow, Russia
[2]Topchiev Institute of Petrochemical Synthesis RAS, Lab. 29, Moscow, Russia
[3]Topchiev Institute of Petrochemical Synthesis RAS, Lab. 14, Moscow, Russia

Backgrounds

Lignocellulose is a most abundant polysaccharide source on the Earth and it is a suitable renewable substrate for the fuel obtaining.

Objectives

Membrane technologies allow to select and to concentrate the liquid fuels (alcohols) from the cultural broths of alcohol and acetone-butanol-ethanol (ABE)-fermentations with lignocellulose enzymatic digests as substrate.

Methods

Lignocelluloses of hard and softwoods as well as grass and topinambur biomass were delignified by environmental-friendly ways, and then subjected to industrial cellulases treatments to obtain C6-sugar's liquors. ABE and bioethanol fermentations were used to produce fuel alcohols which were further concentrated from the broth with membrane technique allowing to get 7-15-times concentrated alcohols with low energy inputs. Resulting concentrated alcohols were used as substrates in the catalytic process for hydrocarbons obtaining (C8-C10) – components of jet fuels:

$2\ ROH + H_2 \rightarrow R–R + 2\ H_2O$, with up to 87% conversion rate.

Viewed drawbacks, restrictions and challenges of the methods exploited will be discussed.

Conclusions

New technology developed to obtain hydrocarbons for jet fuel mixtures manufactured from the renewable natural sources without much damage to the nature in environmentally friendly way without using the fossil resources.

Acknowledgement: this study was supported by grant # 16-14-00098 of Russian Science Foundation.

FEMS7-3206
Biotechnology / Synthetic Biology / Systems Biology - Part III

UNFOLDED PROTEIN RESPONSE (UPR) CAN DRIVE INDUCTION OF IFN-B AND ANTIVIRAL RESPONSES USING A SYNTHETIC PATHWAY BYPASS COMPRISED BY A CHIMERIC XBP1-2CARD-RIG-I PROTEIN.

E. Nistal-Villán[1], R. Ferrero[2], A. Coll[2], J. Argemi[2], S. Rius-Rocabert[1], G. González-Aseguinolaza[2], T. Aragon[2]
[1]Universidad San Pablo CEU-Facultad de Farmacia.,
Secciíón de Microbiología. Dep. de Ciencias Farmacéuticas y de la Salud., Boadilla del Monte, Spain
[2]Center for Applied Medical Research CIMA- University of Navarra.,
Gene Therapy and Regulation of Gene Expression Program, Pamplona, Spain

Backgrounds

Deficiencies in the endoplasmic reticulum (ER) protein folding capacity may cause the activation of signaling mechanisms known as the Unfolded Protein Response (UPR) in eukaryotes. IRE1alpha is an ER-resident transmembrane protein activated by the UPR. Under ER physiological and pathological stress conditions, including viral infections, IRE1alpha activation results in the excision of a non-conventional 26-nucleotide intron within the ORF of the mRNA encoding the transcription factor XBP1, generating a new mRNA that yields the expression of XBP1s protein.

In chordate animals, detection of cytoplasmic viral dsRNA during infections triggers the induction of the IFN-β induction pathway. Its relevance in inducing an antiviral response is highlighted by the many different viral antagonistic mechanisms to prevent such induction.

Objectives

To generate recombinant mRNAs where expression of the retinoic acid inducible gene I (RIG-I) 2CARD domain is controlled by the XBP1 splicing mechanism, bypassing dsRNA detection requirement, in order to stimulate IFN-β-dependent immune responses linked to UPR activation.

Methods

Plasmids containing the minimal XBP1 splicing elements fused to the constitutively active 2CARD domain of the dsRNA sensor RIG-I were constructed. IFN-β induction-dependent reporter, qRT-PCR and antiviral response activities were tested in HEK293T and A549 cells in response to different UPR agonistic stimuli, including viruses in order to measure antiviral response.

Conclusions

We have optimized a minimal XBP1-2CARD-RIG-I sequence to reconstitute the IFN-β pathway in an UPR-dependent fashion. Activation of our system by different agonist suggest the possibility to use unconventional alternative pathways in order to reconstitute type-I IFN deficient systems.

FEMS7-2912
Biotechnology / Synthetic Biology / Systems Biology - Part III

ANTIMICROBIAL ACTIVITY OF THE EXTRACT OF MANILKARA ZAPOTA AGAINST STANDARD MICROORGANISMS FRONTS

A.V. Zaranza[1], A. Ferreira[2], D.H. Silva[2], D. Timóteo[2], J.R. Melo[2], M.B. Camara[3], N. Costa[4], G. Nunes[5], F. Moraes[6], T. Novais[1]
[1]Universidade Federal do Maranhão, Hospital Universitário, São luis, Brazil
[2]Faculdade Estacio São Luís Maranhão, Curso Biomedicina, São luis, Brazil
[3]Instituto Federal de Educação- Ciência e Tecnologia do Maranhão., Química, São luis, Brazil
[4]Universidade Ceuma, Curso Farmácia, São luis, Brunei
[5]Faculdade Estacio São Luís Maranhão, Curso Nutrição, São luis, Brazil
[6]Faculdade Estacio São Luís Maranhão, Curso Enfermagem, São luis, Brazil

Backgrounds

The research of vegetable extracts is important for the discovery of new compounds with therapeutic properties. Among several vegetable species to be investigated the Manilkara zapota (sapoti) stands out. It is a fruit which skin is used by indigenous natives for treatment of diarrhoea and fever.

Objectives

This work has the objective of investigate the antimicrobial activity of the extract of Manilkara zapota.

Methods

The M. zapota (sapoti) fruits were picked at the Estácio São Luís-MA college, under the location 25°28′63,9 ″ S and 44°29′02,6 ″W. The plant registration was obtained in the Herbarium of the Maranhão Federal University, which exsiccate number is 01352. The fruit peel gone through a drying process followed by the production of the extract where it was used solvent extractor hydro alcoholic at 70 % in the proportion of 1:3 (m/v). Next, the extract was subjected to a rotary evaporator under pressure reduced to the controlled temperature of up to 50 °C when it was obtained the final concentration of 0,71mg/mL. Diffusion techniques in ágar and seriated macrodilution were used to evaluate the antimicrobial activity. The following standard vines-strains were used and were originated from the American Type Culture Collection (ATCC): Staphylococcus aureus (25923), Klebsiella pneumoniae (10031), Escherichia coli (25922), Salmonella typhi (14028), Pseudomonas aeruginosa (27853), Candida albicans (14053), Candida krusei (6258), Candida parapsilosis (22019).

Conclusions

The antimicrobial effect of the above mentioned extract against fronts of all the evaluated vine-strains was observed exclusively in the macro dilution technique. It was noted minimal Bactericidal and fungicides concentrations of only 0,08mg/mL and 1,7 mg/mL against samples of P. aerugionsa and C. parapsilosis, respectively.

FEMS7-0776
Biotechnology / Synthetic Biology / Systems Biology - Part III

CONSTRUCTION OF THE CELL SURFACE DISPLAY SYSTEM USING NEW AUTOTRANSPORTER FROM PSYCHROBACTER CRYOHALOLENTIS K5T

K. Novototskaya-Vlasova[1], L. Petrovskaya[2], A. Zlobinov[2], E. Rivkina[3]
[1]Institute of Physicochemical and Biological Problems in Soil Science- Russian Ac, Cryology of soils laboratory, Puschino, Russia
[2]Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry., Bioengineering Department, Moscow, Russia
[3]Institute of Physicochemical and Biological Problems in Soil Science- Russian Academy of Sciences, Cryology of soils laboratory, Puschino, Russia

Backgrounds

Autotransporter (AT) family includes outer membrane proteins from Gram-negative bacteria which consist of N-terminal passenger and C-terminal translocator domains and utilize Type V secretion for export to the cell surface. Previously, we have cloned the gene coding for a potential AT protein (AT877) from *P. cryohalolentis* $K5^T$ isolated from cryopeg within Siberian permafrost. This protein was overexpressed in *Escherichia coli* cells and the passenger domain with lipase activity was displayed on the cell surface.

Objectives

To evaluate applicability of AT877 for the cell surface display of heterologous passengers, we have constructed hybrid proteins which included α-helical linker and translocator domain of AT877. Cold-active esterase EstPc, scaffold protein ^{10}Fn3 (human fibronectin domain) and fluorescent protein mCherry were used as passenger domains.

Methods

Display of the heterologous passengers at the cell surface was confirmed by the use of cell fractionation studies and esterase activity measurements (for EstPc passenger), whole cell ELISA (for ^{10}Fn3) and confocal microscopy (for ^{10}Fn3 and mCherry).

Conclusions

Obtained results prove that the presence of the AT877 α-helical linker and the translocator domain is sufficient for the targeting of various passenger proteins to the surface of *E. coli* cells. This is a new example of the biotechnologically relevant enzyme from the unique microbial community of permafrost and a potential basis for the construction of a new cell-surface display system.

The work was supported by RFBR grant 16-04-00717 and grant SS-8384.2016.4.

FEMS7-1837
Biotechnology / Synthetic Biology / Systems Biology - Part III

PRODUCTION OF 1,2-PROPANDIOL FROM DEOXYSUGARS BY CALDICLLULOSIRUPTOR SPECIES

J. Orlygsson[1], *S. Scully*[1]
[1]*University of Akureyri, Faculty of Natural Resource Sciences, Akureyri, Iceland*

Backgrounds

As 1,2-propanediol (1,2-PD) has a single stereocenter it can be found in two enantiomeric forms. (S)-1,2-PD can be produced microbially with the fermentation of methylpentoses (L-fucose and L-rhamnose) while the (R)-enantiomer is produced from hexoses and pentoses via the methylglyoxal pathway. Few investigations have been reported on thermophiles producing 1,2-PD from deoxysugars although several species within the genus of *Caldicellulosiruptor* have been reported to do so from L-rhamnose

Objectives

The aim of this work is to examine the deoxysugar metabolisms of the whole genus of *Caldicellulosiruptor*.

Methods

In present investigation all nine strains of *Caldicellulosiruptor* are examined under anaerobic conditions in batch culture using serum bottles on glucose, fucose and rhamnose (all 20 mM).

Conclusions

Fermentation of glucose resulted mainly in the production of acetate, lactate and hydrogen but no 1,2-PD was produced, indicating that active methylglyxoal pathway is not present in any of the nine species within the genus. Rhamnose was used as a carbon and energy source for six of the nine species, with values of 1,2-PD ranging from 0.73 mM (*C. owensis*) to 7.7 mM (*C. hydrothermalis*). Other main products were acetate and hydrogen. Fucose was degraded to a mixture of 1,2-PD, acetate, lactate and hydrogen by three (*C. bescii, C. saccharolyticus, C. hydrothermalis*) of the nine species. *C. bescii* produced 3.2 mM of 1,2 PD and *C. saccharolyticus* and *C. hydrothermalis* 8.0 mM. Our results show that the ability of 1,2-PD-production from deoxysugars is more widespread among the genus *Caldicellulosiruptor* than previously reported.

FEMS7-1109
Biotechnology / Synthetic Biology / Systems Biology - Part III

IDENTIFICATION, CLONING AND HETEROLOGOUS EXPRESSION OF A CRYPTIC RIPP GENE CLUSTER FROM THE MARINE STRAIN STREPTOMYCES CANIFERUS

D. Oves-Costales[1], M. Sanchez-Hidalgo[1], J.R. Tormo[2], O. Genilloud[1]
[1]Fundacion MEDINA, Microbiology, Granada, Spain
[2]Fundacion MEDINA, Chemistry, Granada, Spain

Backgrounds

Microbial natural products continue to play an invaluable role in the discovery and development of the chemotherapeutic arsenal. In the last years much attention has been given to marine microorganisms, which have proven to be a rich source of new natural products with therapeutic potential.[1]

Objectives

During our ongoing research with marine actinobacteria we identified by genome mining an intriguing cryptic gene cluster likely encoding the biosynthesis of up to five ribosomally synthesized and post-translationally modified peptide natural products (RiPPs).[2]

Methods

In order to gain more insight into these cryptic metabolites, the gene cluster (17 Kb) was cloned in the integrative pCAP01 vector employing a TAR-based strategy,[3] and the resulting construct was integrated into the host strains *Streptomyces coelicolor* M1152, M1154 and *Streptomyces albus* J1074. These engineered strains were subjected to an array of fermentation conditions, and the corresponding microbial extracts analyzed by LC-MS comparative metabolite profiling in order to identify any cryptic metabolite absent in control fermentations of the host strains.

Conclusions

A combination of genome mining, cloning and heterologous expression was implemented as a strategy to identify new microbial natural products.

References.

1. Gerwick, W, H, Moore, B, S, (2012). *Chem. Biol*. 19 (1):85-98
2. Arnison, P, G, *et al*, (2013). Nat. Prod. Rep. (30): 108-160
3. Yamanaka, K, Reynolds, K, A, Kersten, R, D, Ryan, K, S, Gonzalez, D, J, Nizet, V, Dorrestein, P, C, Moore, B, S, (2014). *Proc. Natl. Acad. Sci. U S A* 111 (5):1957-1962

FEMS7-1099
Biotechnology / Synthetic Biology / Systems Biology - Part III

PURIFICATION AND CHARACTERIZATION OF A-AMYLASE FROM BACILLUS AMYLOLIQUIFACIENS

A. Paloyan[1], L. Melkonyan[2], A. Mkhitaryan[1], A. Hambardzumyan[1], A. Saghyan[3]
[1]SPC "Armbiotechnology" of NAS of Armenia, Laboratory of Molecular Biology, Yerevan, Armenia
[2]SPC "Armbiotechnology" of NAS of Armenia, Laboratory of Selection of BAS Strain-producers, Yerevan, Armenia
[3]SPC "Armbiotechnology" of NAS of Armenia, Director, Yerevan, Armenia

Backgrounds

α-Amylase is used in starch-containing raw material processing and constitute the main masses of enzyme preparations used in biotechnology. In the scientific literature there is described microorganisms application in the field of α-amylase production. The rate of hydrolysis of starch by α-amylase depends on many conditions of the process such as temperature, pH, substrate concentration, enzyme concentration, presence of stabilizing agents.

Objectives

The variety of α-amylases application always drives scientists to search a new α-amylases by improved properties.

Methods

Bacillus amyloliquefaciens strains from the Microbial Depository Center of SPC “Armbiotechnology” NAS RA have been studied as α-amylases sources. The optimum temperature for growth and α-amylase production in the starch-containing media was 45°C. The highest yield of α-amylase activity was after 48 hours of fermentation. α-Amylase of *Bacillus amyloliquifaciens* strain MDC1974 was isolated and purified. After fourth stage of purification (chromatography on DEAE Toyopearl and on hydroxyapatite, 100-10 kDa cut off, gel filtration on Toyopearl 55F and concentration) the specific activity of α-amylase increase 50 times reaching to 350 u/mg. Native gel electrophoresis was used to assess the purity of the enzyme. The degree of homogeneity of α-amylase was exceeded 95%. SDS-PAGE electrophoresis results indicate 58.4 kDa for enzyme molecular weight. α-Amylase shows the highest activity at pH 6.5-7 and at 75°C.

Conclusions

According to the obtained results α-amylase of *Bacillus amyloliquifaciens* has valuable properties and after engineering the strain-producer based on *Bacillus amyloliquifaciens* α-amylase gene will get industrial application.

Acknowledgement:

This work was supported by State Committee Science MES RA, Research Project 16YR-2I016

FEMS7-2149
Biotechnology / Synthetic Biology / Systems Biology - Part III

BIOSYNTHESIS OF PALLADIUM NANOPARTICLES BY PURE AND MIXED CULTURES: KEY PARAMETERS AND MECHANISMS

A. Pat-espadas[1], E. Razo-flores[2], R. Rangel-mendez[2], F.J. Cervantes[2]
[1]Geology Institute Unam, Northwest Regional Station, Hermosillo, Mexico
[2]Instituto Potosino De Investigacion Cientifica Y Tecnologica, Environmental Sciences, San Luis Potosi, Mexico

Backgrounds

Nanoparticles are extensively used because they exhibit unique electronic, magnetic, catalytic and optical properties which are different from those of bulk materials. The use of microorganisms for remediation purposes has been applied since years ago; however, the approach of production of nanoparticles is very recent. Biological methods constitute a feasible option that also offer advantages over traditional methods of synthesis.

Objectives

The aim of this work was to study the influence of different parameters such as metal and cells concentration, time and electron donor on the synthesis of palladium nanoparticles by pure and mixed cultures.

Methods

Pure culture of *Geobacter sulfurreducens* strain PCA (DSM 12127; ATCC51573) was routinely cultured anaerobically in acetate:fumarate medium and exposed to concentrations of 25, 50 and 100 mg Pd(II)/L as Na_2PdCl_4.

Anaerobic granular sludge was explored for its ability to reduce Pd(II) to Pd(0). For this six different electron donors were explored. Control assays were carried out in cell-free media. All assays were incubated at 30° C and performed in duplicate. Samples were analyzed at specific time intervals. Analytical techniques such as transmission electron microcopy, FT-IR spectra and X-ray diffraction were used.

Conclusions

The results obtained from this study demonstrated that the biosynthesis of nanoparticles is mainly influenced by: speciation (soluble and solid species), metal and cell concentrations, electron donor and the presence or absence of a redox mediator.

In pure cultures the results obtained demonstrated that NPs synthesis occur by means of two mechanisms: (i) direct contact involving reduction by outer membrane c-type cytochromes; and (ii) indirectly by using an electron shuttle. For mixed cultures the reduction of Pd(II) is strongly dependent on the electron donor used and inhibition occurs at levels as low as 0.96 mg Pd(II)/L. However it is possible to achieve Pd reduction by setting the adequate parameters of synthesis.

FEMS7-1075
Biotechnology / Synthetic Biology / Systems Biology - Part III

BUTANOL TOLERANCE VERSUS BUTANOL PRODUCTION IN MUTANTS AND ADAPTED STRAINS OF CLOSTRIDIUM BEIJERINCKII

P. Patakova[1], M. Vasylkivska[1], J. Kolek[1], M. Drahokoupil[1]
[1]University of Chemistry and Technology Prague, Department of Biotechnology, Prague 6, Czech Republic

Backgrounds

Butanol production is a unique feature of so-called solventogenic clostridia which belong to heterofermentative, Gram-positive, sporulating bacteria performing acetone-butanol-ethanol (ABE) fermentation. Unfortunately, the main bottleneck of the process, which prevents its industrial application, is low final butanol concentration caused by poor butanol tolerance of the production strains.

Objectives

To overcome this problem, different mutation and adaptation techniques were tested to obtain more tolerant strains (in comparison to parent *Clostridium beijerinckii* strain). The strains exhibiting more tolerant phenotype were further used for butanol production.

Methods

For obtaining mutant strains, chemical mutagenesis using ethyl methanesulfonate (EMS) was used. The obtained mutants were grown in media containing increasing butanol concentration using bromcresol purple as growth indicator, because vegetative multiplication of *C.beijerinckii* is associated with acids production. In addition, butanol adapted strains were prepared by repeated re-inoculation of vegetative cells or spores to media with increasing butanol concentration. The strains exhibiting the most tolerant phenotype were further tested for butanol production under substrate non-limiting conditions i.e. in media containing high sugar concentration.

Conclusions

High tolerance and high production features are rarely present together. However mutant and adapted strains showing both these properties will be a subject of further investigation which will be focused on finding the cause of this win-win situation.

Acknowledgement
This work has been supported by grant project GACR 17-00551S.

FEMS7-1534
Biotechnology / Synthetic Biology / Systems Biology - Part III

NEW COLORIMETRIC ASSAY FOR HYALURONIC ACID DETERMINATION IN COMPLEX SAMPLES

S. Pepeliaev[1], D. Smirnou[1], J. Jana[1], F. Lukáš[1], Č. Zbyněk[1], V. Vladimír[2]
[1]Contipro A.S., Fermentation group, Dolní Dobrouč, Czech Republic
[2]Contipro A.S., CEO, Dolní Dobrouč, Czech Republic

Backgrounds

Hyaluronic acid (HA) is a unique biopolymer widely used in medicine and cosmetics. The knowledge of precise HA concentration is a must in pharmaceutical and cosmetic production as well as in diagnostics. There are several groups of methods routinely used for HA quantification each having its own advantages and drawbacks. The right choice of an appropriate method is based on HA concentration range, complexity of sample and good understanding of principles of the given method.

Objectives

Introduction of a new colorimetric assay for HA determination and its comparison with already existing methods. Selection of the most appropriate method depending on application.

Methods

New enzyme-coupled colorimetric assay with high sensitivity and range based on HA digestion with Streptococcus pneumoniae hyaluronan lyase SpnHl and subsequent colour reaction of unsaturated disaccharides with 3-methyl-2-benzothiazolinone hydrazone. Comparison of the new assay with already known methods based on turbidimetry, colorimetry, immunoprecipitation, gravimetry, high performance liquid chromatography, gel electrophoresis.

Conclusions

Newly presented assay for HA determination offers the highest sensitivity among the known colorimetric methods, is reasonably fast, safe and inexpensive. The assay has modular structure and thus is very flexible: it could be used for a variety of samples detecting HA in range 1-2000 µg/ml. The presented assay could replace other methods in applications not demanding nanogram-scale sensitivity. Such applications are yield control in biotechnological production of HA, quality control of HA-containing pharmaceuticals and cosmetics, HA content determination in skin, joints, vitreous humour and other tissues and organs relatively rich in HA. For nanogram-scale application the ELISA-based assays are recommended.

FEMS7-1490
Biotechnology / Synthetic Biology / Systems Biology - Part III

NOVEL AND UNFORESEEN TRAITS OF THE REGULATORY CIRCUIT MASTERED BY DNTR, THE REGULATOR OF 2,4-DINITROTOLUENE BIODEGRADATION IN BURKHOLDERIA CEPACIA R34

D. Perez-Pantoja[1], P. Nikel[2], M. Chavarria[3], V. de Lorenzo[4]
[1]Universidad Tecnológica Metropolitana, Programa Institucional de Fomento a la I+D+i, Santiago, Chile
[2]Technical University of Denmark, The Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark
[3]Universidad de Costa Rica,
Escuela de Química & Centro de Investigaciones en Productos Naturales CIPRONA, San José, Costa Rica
[4]Centro Nacional de Biotecnología CNB-CSIC, Programa de Biología de Sistemas, Madrid, Spain

Backgrounds

B. cepacia R34 mineralizes the xenobiotic compound 2,4-dinitrotoluene (DNT) owing to the dnt genes encoding the Rieske-type (RTDO) DNT dioxygenase (DNTDO) that are transcriptionally controlled by the LysR-type DntR regulator.

Objectives

In this work we addressed for the first time the regulatory network mastered by DntR revealing several traits previously unnoticed in the multiple studies performed in surrogate hosts.

Methods

For an accurate assessment of the regulatory role of substrates and enzymes implied in this system we first evaluated the suitability of naphthalene as a DNTDO substrate and its deleterious effect over host physiology. Experiments of substrate consumption and oxidative stress revealed that naphthalene is a DNTDO substrate as good as DNT but its metabolization is much less stressful for the native host. We accomplished the generation of a dntR mutant derivative and confirmed its role as the exclusive activator of DNTDO-encoding genes in response to salicylate. The detailed description of the regulatory system commanded by DntR was performed by using gfp transcriptional fusions to the PdntA promoter and the previously uncharted PdntR promoter, revealing a striking positive autoregulation by this activator. The utilization of naphthalene in addition to salicylate as inducer revealed the requirement of a DNTDO substrate for full activation of both promoters, thereby counteracting any gratuitous induction. Additionally, a dntAa mutant derivative revealed that a strain harbouring an inactivated DNTDO showed maximal induction by salicylate in both promoters irrespective of naphthalene presence. This suggested that the dioxygenase is able to block the activation by DntR and that the presence of a substrate would release such a repression.

Conclusions

The regulatory circuit described in this work suggests that communication between the activity of RTDO and the system controlling their expression is a key trait that explains the widespread presence of this conspicuous enzymatic family in routes evolved for degradation of xenobiotics.

FEMS7-2731
Biotechnology / Synthetic Biology / Systems Biology - Part III

SYNTHETIC ADHESINS TO TARGET ENGINEERED E. COLI TO HUMAN TUMORS EXPRESSING EGFR

C. Mañas[1], E. Pico[1], L.Á. Fernández-Herrero[1]
[1]Centro Nacional de Biotecnología CNB-CSIC, Biotecnología microbiana, Madrid, Spain

Backgrounds

E. coli and other anaerobic bacteria can colonize solid tumors. We have previously developed a way to improve the colonization ability of *E. coli* to solid tumors by engineering the expression on the bacterial surface of synthetic adhesins (SAs) that bind to specific surface antigens expressed on tumor cells [1]. SAs are based on single domain antibodies (nanobodies) fused to the β-domain of Intimin. This technology was originally tested *in vitro* and *in vivo* using SAs binding GFP, which was expressed on the surface of HeLa tumor cells.

Objectives

Apply the SAs technology into relevant human tumor models naturally expressing a validated surface tumor antigen, such as the epidermal growth factor receptor (EGFR). Bladder and colon cancers are adequate models since EGFR is frequently overexpressed by these tumor cells and these organs represent a good environment for *E. coli*.

Methods

We have generated SAs against EGFR based on our previous selection of nanobodies binding EGFR [2]. SAs of higher affinity and specificity were integrated in the chromosome of *E. coli* and their expression and binding to EGFR was confirmed by flow cytometry analysis. The engineered *E. coli* strains with these SAs bind specifically to bladder and colon carcinoma cell lines expressing EGFR.

Conclusions

We have generated SAs against human EGFR that allow the specific binding of *E. coli* to human bladder and colon carcinoma cells expressing this tumor antigen.

References

1. Piñero-Lambea, C., et al., ACS Synth Biol, 2015. **4**:463-73.
2. Salema, V., et al., MAbs, 2016. **8**:1286-1301.

FEMS7-2003
Biotechnology / Synthetic Biology / Systems Biology - Part III

TIME-RESOLVED TRANSCRIPTOMICS AND CONSTRAINT-BASED MODELLING IDENTIFY SYSTEM-LEVEL METABOLIC FEATURES AND OVEREXPRESSION TARGETS TO INCREASE SPIRAMYCIN PRODUCTION IN STREPTOMYCES AMBOFACIENS

E. Pinatel[1], M. Fondi[2], T. Adelfia[3], F. Damiano[3], C. Consolandi[1], B. Mattorre[2], D. Fico[4], G.E. De Benedetto[4], L. Siculella[3], G. De Bellis[1], P. Alifano[3], C. Peano[1]
[1]National Research Council, Institute of Biomedical Technologies, Segrate, Italy
[2]University of Florence, Computational Biology Group, Sesto Fiorentino, Italy
[3]University of Salento, Department of Biological and Environmental Sciences and Technologies, Lecce, Italy
[4]University of Salento,
Laboratory of Analytical and Isotopic Mass Spectrometry- Department of Cultural Heritage, Lecce, Italy

Backgrounds

The metabolic pathways leading to industrially relevant secondary metabolites production, like antibiotics, usually compete for pools of metabolic intermediates common to the biosynthetic processes necessary for bacterial growth. *In silico* modelling and omic-approaches are becoming crucial to provide system level comprehension of bacterial metabolism, enabling the identification of new strategies to obtain industrially relevant engineered strains.

Objectives

In this study we characterized the metabolic landscape of *Streptomyces ambofaciens,* an industrial producer of antibiotics, anti-fungal and iron-chelating compounds, to predict new metabolic engineering targets for their overproduction.

Methods

We took advantage of the recently published *S. ambofaciens* genome sequence and of databases for gene/protein function prediction to reconstruct its metabolic model,manually refining it to improve the accuracy of growth rate predictions and to include antibiotic production pathways. The integration of time-resolved transcriptomic data with Flux Balance Analysis was then adopted to highlight the metabolic adjustments occurring in different growth conditions. At the same time Flux Scanning based on Enforced Objective Flux (FSEOF) analysis was performed to identify new targets for antibiotic production increase.

Conclusions

A metabolic model of *S. ambofaciens,* including 1244 genes and spyramicin, antimycin, stambomycin and congocidine complete pathways, was produced, and it represents a solid platform for the exploitation of *S. ambofaciens* biotechnological potential. Then, through different data integration, we outlined the main effects of gene expression changes on the overall metabolic reprogramming occurring during *S. ambofaciens* growth curve. Moreover, we identified a set of new potential overexpression target candidates for spiramycin over-production and experimentally validated one of them.

FEMS7-2270
Biotechnology / Synthetic Biology / Systems Biology - Part III

ESCHERICHIA COLI GROWTH AND HYDROGEN PRODUCTION USING BREWERY WASTE: OPTIMAL PRETREATMENT AND ROLE OF HYDROGENASES

A. Poladyan[1], *K. Trchounian*[1], *A. Trchounian*[1]
[1]*Yerevan State University, Department of Biochemistry- Microbiology and Biotechnology, Yerevan, Armenia*

Backgrounds

Dihydrogen (H_2) is a clean, renewable energy for the future. Brewery spent grains (BSG), one of the by-products of brewery production, were applied for *Escherichia coli* growth and H_2 production.

Objectives

E. coli BW25113 wild-type and hydrogenase (Hyd) mutants with deletions of genes for key subunits of Hyd1-4 (Δ*hyaB,* Δ*hybC,* Δ*hycE,* Δ*hyfG*), respectively, as well as Δ*hyaB*Δ*hybC* double mutant growth, redox potential kinetics and H_2 production were investigated upon BSGH utilization. After 24 h growth mutants biomass yields were ~0.3 g dry weight L^{-1}, closed to wild type value, and ~1.8 fold less compared with the data of wild type bacteria grown on peptone medium (PM) with glycerol. However, specific growth rates were similar, even ~1.1 fold stimulated upon Δ*hyaB*Δ*hybC* double mutation. Readings of redox Pt electrode dropped up to -400 ±10 mV, with H_2 yield of 0.75 mmol/L at the 3rd h of wild type growth and persisted until 48 h. Whereas, redox Ti-Si electrode readings drop was negligible and kept positive in contrast to glycerol fermentation in PM. H_2 production was not observed with defective Hyd3 and Hyd4, therefore, Hyd3 and Hyd4 are responsible for H_2 production using BSGH, whereas defective Hyd1 and Hyd2 led to up to 2 fold stimulation of H_2 yield.

Methods

The dilute acid and alkali pretreatment methods were used to hydrolyze the lignocellulose structure; and the BSG hydrolysate (BSGH) optimal conditions for bacterial growth and H_2 production were designed.

Conclusions

These findings might be useful for development of H_2 production biotechnology using different organic wastes.

FEMS7-2891
Biotechnology / Synthetic Biology / Systems Biology - Part III

BIOTRANSFORMATION AND VALORIZATION OF RESIDUES AND WASTES BY FUNGAL LIPASES

M. Molina-Gutiérrez[1], N.L.S. Hakalin[1], M.D. Moldovan[1], L. Rodríguez-Sánchez[2], M.J. Martínez[1], A. Prieto[1]
[1]Centro de Investigaciones Biológicas CIB-CSIC, Environmental Biology, Madrid, Spain
[2]Centro de Investigaciones Biológicas CIB-CSIC, Gas Chromatography, Madrid, Spain

Backgrounds

Across the last decade, the use of lignocellulosic wastes for bioethanol production has been promoted, as they constitute a renewable, available and inexpensive feedstock that does not compete with food production. Industrial manufacture of 2G-bioethanol is already a fact, but to yield a profitable process, not only the polysaccharides from plant biomass must be exploited. The lipid fraction of some residues may contain interesting components to be used for industrial purposes upon their appropriate transformation, for example, in esters. These esters are generally produced by chemical catalysis, but most of these reactions can also be performed by clean and soft procedures, using biocatalysts.

Objectives

Commercial preparations with lipase or sterol esterase activities, and the versatile lipase secreted by *Ophiostoma piceae* (OPE), were compared as catalysts of the synthesis of esters of biotechnological interest using industrial residues as lipid sources. The enzymes were immobilized and their effectiveness and recyclability compared.

Methods

Lipid fractions were extracted from biomass and used as substrates for enzymatic conversion by CalA, CalB or OPEr. Reactions were developed at 28 °C in a vertical mixer. The reaction products were analyzed by TLC or GC. Enzymes were covalently immobilized onto silanized magnetic nanoparticles (MNPs) as mCLEAs or by specific linkage to amino-functionalized-MNPs.

Conclusions

Fatty acid-esters of phytosterols, hydroxycinnamic acids, and short-chain alcohols can be synthetized using the assayed fungal lipases, although with different efficiency. Enzyme immobilization onto MNPs allows easy recovery of the catalyst for further recycling.

Thanks to MINECO BIO2015-68387-R and CM S2013/MAE-2907 for funding.

FEMS7-0300
Biotechnology / Synthetic Biology / Systems Biology - Part III

A NOVEL PROGRESSIVE CLUSTERING APPROACH FOR CREATING ECOLOGICAL NETWORKS REVEALS THE STRUCTURE OF MICROBIAL COMMUNITIES AT DIFFERENT SCALES

F. Puente-Sánchez[1], J. Tamames[1]
[1]Centro Nacional de Biotecnología-CSIC, Systems Biology, Madrid, Spain

Backgrounds

Usual methods for building ecological networks are based on co-occurrences between two taxa. But since microbial communities are probably structured by interactions between multiple partners, these approaches are likely to miss most of the complexity of natural microbiomes.

Objectives

Our main goal was to design a procedure capable to identify aggregations of taxa that can constitute building blocks nucleating the whole community, and characterise them from the ecological and functional point of view.

Methods

We have devised a new method for creating networks, in which a progressive, agglomerative clustering procedure is used to recover modules of co-occurring organisms. The modules have different size, from two taxa to tens of them. We have used the samples in microDB database (http://botero.cnb.csic.es/envDB), comprising more tan 20.000 environmental samples from all kind of environments. The usage of this huge set of information required the development of a new scoring measure adapted to the presence/ausence data characteristic of this database.

Conclusions

We have found specific modules for each environment, but also many that can be shared. These modules act like a nucleating unity to which other taxa can adhere to form progressively bigger communities. We have characterized these paths of aggregation using the genome content of the taxa on them, studying the metabolic similarity or divergence of the newly recruited taxa. Thus we are able to study patterns of functional redundancy and complementation, finding that redundancy is more prevalent. We also show the correlation between particular metabolic pathways and niche specialization.

FEMS7-2831
Biotechnology / Synthetic Biology / Systems Biology - Part III

MULTI-OMICS OF PSEUDOALTEROMONAS HALOPLANKTIS TAC125: A QUEST FOR ANTIMICROBIAL METABOLIC PATHWAYS

M. Fondi[1], E. Perrin[1], A. Fiore[2], G. Diretto[2], I. Maida[1], A. Sutera[3], G. Renzone[4], G. Gallo[3], A. Scaloni[4], A.M. Puglia[3], C. Barbara[5], L. Carraro[5], R. Fani[1]
[1]University of Florence, Dep. of Biology, Florence, Italy
[2]Ente Nazionale per le Nuove Tecnologie- l'Energia e l'Ambiente, Dip. Innovazione-Divisione Biotecnologie e Agricoltura, Rome, Italy
[3]University of Palermo, Dip. STEBICEF, Palermo, Italy
[4]National Research Council, ISPAAM, Naples, Italy
[5]University of Padova, Dep. of Comparative Biomedicine and Food Science, Padova, Italy

Backgrounds

The Antarctic strain *Pseudoalteromonas haloplanktis* TAC125 is one of the model organisms of cold-adapted bacteria and is currently exploited as a new alternative expression host for numerous biotechnological applications. Interestingly, this bacterium has been reported to be able to inhibit the growth of *Burkholderia cepacia* complex (Bcc) strains, opportunistic pathogens responsible for the infection of immune-compromised patients. Most likely, this occurs through the synthesis of several different compounds, including Volatile Organic Compounds (VOCs), whose nature and characteristics are currently mostly unknown.

Objectives

To obtain a complete picture of cellular processes differentially regulated and associated with the capability of inhibiting Bcc growth.

Methods

Transcriptomic, proteomic and metabolomic experiments were carried out on *P. haloplanktis* TAC125 grown using two different cultivation media in which the strain is able to inhibit or not Bcc growth.

Conclusions

Bcc growth inhibition capability is deeply linked to the medium used to cultivate *P. haloplanktis* TAC125. Therefore, multi-omic data integration was used in order to explain the emergence of *P. haloplanktis* TAC125 phenotypes and, specifically, of cellular functional states associated to capability of inhibiting Bcc growth. In perspectives, the design of more focused strategies for a rational biotechnological exploitation of this strain will be boosted by the approach used and the results obtained in this work.

FEMS7-2308
Biotechnology / Synthetic Biology / Systems Biology - Part III

PRODUCTION OPTIMIZATION OF AN ANTICANCER COMPOUND, PRODIGIOSIN, FROM SERRATIA SP. S2B AND DESIGN OF ITS DRUG DELIVERY SYSTEM TO TARGET CELLS, USING NANOCARRIER

B. Rastegari[1], H.R. Karbalaei-Heidari[1], S. Zeinali[2], S. Heather[3]
[1]Shiraz University, Biology, Shiraz, Iran
[2]Shiraz University, Nanochemical engineering, Shiraz, Iran
[3]McMaster University, Chemical engineering, Hamilton, Canada

Backgrounds

Among several thousands of microbial secondary metabolites, the prodiginine family is considered as an antimicrobial, antimalarial, immunosuppressive, and remarkable anticancer agent - The selective cytotoxicity effect on malignant cell lines has motivated researchers to introduce them as a potent anticancer drug The main drawback of prodigiosin like other poor water soluble drugs is its limited bioavailability therefore, must be bound to biocompatible polymers to remain in its effective concentration in blood.

Objectives

Production optimization of the prodigiosin from a newly isolated bacterium, *Serratia* sp. S2B and its introduction as a selective anticancer drug to breast and hepatic cancerous cells by the design of protein based nanocarriers and polysaccharide coated magnetic nanoparticles.

Methods

A new bacterial strain S2B which produces prodigiosin was isolated and characterized as *Serratia* genus via 16S rDNA sequencing. Pigment production was optimized by assessment of different carbon, nitrogen sources and physicochemical factors effects using statistical approaches. Furthermore, the interaction of prodigiosin with human serum albumin and β- lactoglobulin as intravenous and oral scaffolds was studied by fluorescence, circular dichroism spectroscopy and molecular docking methods. Finally, fabrication of magnetite nanoparticles using chitosan and β-cyclodextrin was conducted to design an active targeting system.

Conclusions

Pigment production was optimized using low-cost medium containing sugar beet molasses and ammonium sulfate. The sulfate was introduced as an important factor in regulation of pigment biosynthesis pathway. Both tested protein nanocarriers as well as chitosan coated magnetic nanoparticles can be introduced as smart drug delivery system for breast and hepatic cancer chemotherapy

FEMS7-2171
Biotechnology / Synthetic Biology / Systems Biology - Part III

SMALL RNAS ACTIVATED BY THE PHOSPHATE STARVATION RESPONSE REGULATOR PHOP IN STREPTOMYCES

S. Martín-Martín[1], M. Ordóñez-Robles[1,2], F. Santos-Beneit[1,3], A. Sola-Landa[1], R. Pérez-Redondo[1], J.F. Martín[2], A. Rodríguez-García[1,2]
[1]Asociación de Investigación INBIOTEC Instituto de Biotecnología de León, Área de Genómica, León, Spain
[2]Universidad de León, Área de Microbiología- Departamento de Biología Molecular, León, Spain
[3]Center for Bacterial Cell Biology, Newcastle University, Newcastle, United Kingdom

Backgrounds

Streptomycetes are bacteria of high biotechnological relevance due to their diverse secondary metabolite production. Secondary metabolites are produced only under some nutritional conditions, e.g., phosphate starvation. As occurs in other bacteria, in *Streptomyces* the two-component system PhoR-PhoP triggers the response to phosphate limitation. PhoR, the sensor kinase, phosphorylates and, hence, activates the response regulator PhoP. The number of two-component systems that contain sRNAs (small RNAs) in their regulons is growing. sRNAs can inhibit or activate the expression of several target genes.

Objectives

Our general objective was to explore the role of sRNAs on the response to phosphate starvation mediated by the PhoR-PhoP two component system in *Streptomyces*.

Methods

Parental strain M145 and Δ*pho*P mutant strain INB201 of *Streptomyces coelicolor* were cultured in flasks containing defined MG medium. Samples were taken from 36 h to 42 h of culture, when phosphate becomes depleted from the medium and the *pho* regulon is activated. The transcriptomes of both strains were analyzed using dRNA-seq and bioinformatically predicted transcripts served to design Agilent 4x44k microarrays. Then, RNA samples were chemically label to avoid retrotranscription artefacts. Microarray profiles were analyzed as previously (PMID: 17623301).

Bioinformatics searches predicted putative PhoP binding sites in sRNA promoters. Gel-retardation and DNase I footprinting analyses were used to validate PhoP binding.**Conclusions**

A total of 74 sRNAs were identified as putative members of the *pho* regulon. Binding assays confirmed that the response regulator PhoP directly activates at least four of them.

FEMS7-2287
Biotechnology / Synthetic Biology / Systems Biology - Part III

OMICS ANALYSES OF PHYTOSTEROLS-INDUCING DIFFERENTIAL EXPRESSION IN 'MYCOBACTERIUM NEOARUM' NRRL B-3805, A PRODUCER OF THE STEROIDS PRECURSOR ANDROSTENEDIONE

I. Vázquez-Iglesias[1], A. Morales[1], P. González-Álvarez[2], M. Castro-Mariñas[2], A. Sola-Landa[2], A. Rodríguez García[2], C. Barreiro[1]
[1]Asociación de Investigación INBIOTEC Instituto de Biotecnología de León, Área de Proteómica, León, Spain
[2]Asociación de Investigación INBIOTEC Instituto de Biotecnología de León, Área de Genómica, León, Spain

Backgrounds

The microbial bioconversion of phytosterols into steroid precursors is an industrial process that allows the economical production of steroid drugs. Phytosterols are a cheap source of the steroid carbon skeleton. '*Mycobacterium neoaurum'* NRRL B-3805 is a UV-mutant of a soil isolate that efficiently degrades the phytosterols side chain and produces the steroid precursor 4-androstene-3,17-dione (AD). The availability of the complete genome sequence of this strain, recently published by the group (PMID: 26988397), allows the development of transcriptomics and proteomics analyses.

Objectives

To identify key bioconversion genes for further improvement of the strain by means of a differential expression comparison between cells cultured in media with or without added phytosterols.

Methods

Cells were cultured in defined medium containing glycerol or glycerol plus sterols as the carbon sources. 2D-DIGE proteome analyses (intra- and extra-cellular) were performed and proteins were subsequently identified by means of a MALDI-TOF/TOF mass spectrophotometer. RNAseq data served to identify transcribed regions and then to design probes for differential transcriptomics with Agilent 8x15k microarrays. Metabolic pathway analysis were carried out using the BioCyc web server, which allowed the identification of pathways involved in sterols bioconversion.

Conclusions

The analysis of the results showed expression profiles clearly conditioned to the sterols addition, as well as, sterols dependent proteins/genes, which suggest a sterol specific response.

FEMS7-1790
Biotechnology / Synthetic Biology / Systems Biology - Part III

A DNA METHYLATION-DEPENDENT SWITCH FOR THE CONSTRUCTION OF BIOLOGICAL SENSORS

D. R. Olivenza[1], *I. Cota*[1], *J. Casadesús*[1]
[1]*Universidad de Sevilla, Departamento de Genética, SEVILLA, Spain*

Backgrounds

The *Salmonella enterica ovpAB* operon encodes inner membrane proteins that control O-antigen chain length. Expression of *opvAB* undergoes phase variation under transcriptional control by Dam methylation and OxyR.

Objectives

We intend to use the OpvAB phase variation system for the construction of artificial toggle switches with potential uses as biological sensors.

Methods

A ~700 bp DNA fragment containing the *opvAB* promoter and the upstream control region is used to drive phase-variable transcription of reporter genes (e. g., the *E. coli lac* operon and the green fluorescent protein gene, *gfp*). The constructions are active in both *S. enterica* and *E. coli*.

Conclusions

1. A strain carrying a *gfp* fusion downstream of *opvAB* provides a highly sensitive sensor of Dam methylase inhibition. The size of the ON subpopulation permits quantitative assessment of the inhibition (measured by either fluorescence intensity or β-galactosidase activity). A sensor of this kind can be useful to search for drugs that inhibit DNA methylation.

2. OpvAB^{OFF} cells are sensitive to bacteriophages that target the O-antigen while OpvAB^{ON} cells are resistant. In the presence of a bacteriophage, OpvAB^{ON} cells take over the culture. The presence of a bacteriophage can thus be detected by an increase in fluorescence (if a *gfp* fusion is used) or in β-galactosidase activity (if a *lac::gfp* fusion is used). A sensor of this kind can be useful to monitor the presence of bacteriophages (and, in an indirect manner, to detect the presence of bacteriophage-sensitive bacterial cells).

FEMS7-2875
Biotechnology / Synthetic Biology / Systems Biology - Part III

BACTERIAL COMPUTATION USING PLASMIDS AS WIRES: POWERING THE MACHINE

R. Fernandez Lopez[1]
[1]*IBBTEC, Molecular Biology, Santander, Spain*

Backgrounds

Biological computation is a fundamental topic on synthetic biology. Usually the approach is to design gene regulation networks able to modulate reporter genes in response to chemical or physical inputs.

This approach poses some problems, specially related to scaling the computing system:

- Diffusible chemical signals are non-directional.

- There are few Input-regulator pairs available .

- Gene regulation is a stochastic process with big fluctuations associated.

Objectives

To address these issues we propose bacterial conjugation. It is a directional, modular, and binary system of communication, computing modules can be used as much as they are needed in a network and there are plenty of different orthogonal channels described (~50 relaxase-oriT pairs).

Bacterial conjugation consists on the cut and transport of a bacterial plasmid from a bacterium to another in contact using a Type IV Secretion System. Conjugative plasmids carry all the necessary genes. When these plasmids do not carry the secretion system genes, they are called *mobilizable*. As every gene involved in the process is essential, they can be complemented in *trans* (allowing conditional operations and building of AND gates).

Methods

In this work we first characterized conjugation of model plasmid R388 (40kb, IncW) and found an intrinsic limitation to the conjugation efficiency (number of plasmid transmissions per donor cell and per generation); as long as we need a highly efficient communication system, the original system was modified in order to increase this conjugation rate. For this purpose we used a systems biology approach, tuning gene expression of T4SS components.

Conclusions

. .

FEMS7-2407
Biotechnology / Synthetic Biology / Systems Biology - Part III

THE ROLE OF 3-HYDROXY-3-METHYLGLUTARYL COA REDUCTASE OF USTILAGO MAYDIS (HMGRUM) AND A PROPOSED RECOMBINANT ENZYME (REC-HMGRUM) TO TEST ANTIFUNGAL COMPOUNDS AND LIPID-LOWERING

B. Rosales Acosta[1], M.D.L. Villa Tanaca[1], J.A. Ibarra Garcia[1], C.H. Hernandez Rodriguez[1]
[1]Escuela Nacional de Ciencias Biológicas - Instituto Politécnico Nacional, MICROBIOLOGY, México, Mexico

Backgrounds

The enzyme 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMGR), catalyzes the synthesis of mevalonate from 3-hydroxy-3-methylglutaryl CoA, the key compound in the synthesis of cholesterol in humans and ergosterol in fungi. HMGRs have been widely studied in fungi because they share structural similarity with those from mammals. Amongst the most studied HMGRs are those from a *Saccharomyces cerevisiae*, *Saccharomyces pombe*, *Candida glabrata* and *Candida albicans*.

A phylogenetic analysis multiple HMGR from different organisms showed that the HMGR from human chas a higher similarity with that from *Ustilago maydis* than with other fungal HMGRs. Currently, several drugs have been designed derivatived from statins for using as hypolipemc that inhibit HMGR activity and hence cholesterol synthesis. System models used to test these drugs range from rat liver extracts to the use of a recombinant human HMGR, buts this latter is a hazle as it is hard to acquire the latter as it is the product of a human gene.

Objectives

To evaluate the effect of specific inhibitors of the enzyme 3-hydroxy-3-methylglutaryl CoA reductase from *Ustilago maydis* to know the role that this enzyme plays in the fungus life cycle

Methods

Using in silico analysis, the ORF codifying for HmgrUm wasidentified and the protein characteristics were deduced. The effect ofthe competitive inhibitors of HmgrUm on the viability of thisbasidiomycota, the synthesis of its sterols, and its mating wereevaluated.

Conclusions

In conclusion, the HmgrUm is proposed as a study model to test lipid-lowering and antifungal compounds

FEMS7-1124
Biotechnology / Synthetic Biology / Systems Biology - Part III

DIRIGENT PROTEINS AS VERSATILE TOOLS IN BIOTECHNOLOGY

J.F. Rosengarten[1], K.R. Hayes[2], J. Pietruszka[3], M. Holz[2], T. Classen[1]
[1]Institut für Bio- und Geowissenschaften IBG-1- Forschungszentrum Jülich GmbH, Chemie, Jülich, Germany
[2]Institut für Bioorganische Chemie der Heinrich-Heine-Universität Düsseldorf im Forschungszentrum Jülich, Chemie, Jülich, Germany
[3]Institut für Bio- und Geowissenschaften IBG-1- Forschungszentrum Jülich GmbH / Institut für Bioorganische Chemie der Heinrich-Heine-Universität Düsseldorf im Forschungszentrum Jülich, Chemie, Jülich, Germany

Backgrounds

The introduction of regio- and stereoselectivity to reactions does often go along with many efforts in the course of chemical synthesis. Dirigent Proteins (DPs) are promising tools used by nature to mediate selectivity towards usually unselective laccase-catalyzed reactions. The reaction for which DPs have been initially described is the coupling of two coniferyl alcohol radicals to yield optically pure (+)- or (-)-pinoresinol during lignan biosynthesis (1, 2, 3, 4).

Objectives

Although research in the field of DPs is ongoing since the first member of the DP family has been presented in 1992 (5), there is still much space left for their deeper investigation. Hereby, one of the main challenges is their soluble expression in the common lab workhorse *Escherichia coli*, as formation of inclusion bodies has been observed (4).

Methods

In our work we have generated an expression system allowing the recombinant production of the prominent Dirigent Protein AtDIR6 from *Arabidopsis thaliana* in a soluble form in bacteria. We are aiming at the detailed study of the molecular mechanisms of these bacterially produced DPs as well as at their application for synthetic purposes.

Conclusions

Our results give first evidence for the bacterial production of recombinant DPs in a soluble form and in scale allowing for in-depth studies of the purified protein.

1 Davin *et al*. (1997). *Science*. **275**(5298): 362-6

2 Davin and Lewis (2005). *Curr Opin Biotechnol*. 16(4): 398-406

3 Pickel *et al*. (2010). *Angew Chem Int Ed Engl*. **49**(1): 202-4

4 Pickel and Schaller (2013). *Appl Microbiol Biotechnol*. **97**(19): 8427-38

5 Davin *et al*. (1992). *Phytochemistry*. **31**(11): 3869-3874

FEMS7-1385
Biotechnology / Synthetic Biology / Systems Biology - Part III

ROLE OF TRANSCRIPTION ACTIVATOR CAT8 IN REGULATION OF XYLOSE METABOLISM AND ALCOHOLIC FERMENTATION IN THE THERMOTOLERANT YEAST OGATAEA POLYMORPHA

J. Ruchala[1], O. Kurylenko[2], K. Dmytruk[2], A. Sibirny[1,2]
[1]University of Rzeszow,
Faculty of Agriculture and Biology- Department od Biotechnology and Microbiology, Rzeszów, Poland
[2]Institute of Cell Biology- NAS of Ukraine, Department of Molecular Genetics and Biotechnology, Lviv, Ukraine

Backgrounds

Ogataea polymorpha is thermotolerant yeast capable of glucose, cellobiose and xylose fermentation, however, ethanol yield from xylose is very low. Several approaches have been developed for construction of the advanced ethanol producers from xylose; still ethanol yield and productivity from xylose have to be further improved. Till now, nobody studied the role of transcription activators in regulation of xylose metabolism and fermentation. We paid attention on Cat8 transcription activator involved in regulation of gluconeogenesis and catabolism of alternative carbon sources in *Saccharomyces cerevisiae*.

Objectives

We aimed to construct strains with deletion and overexpression of *CAT8* gene on background of the wild type and the best available ethanol producer of *O. polymorpha* and study the effects of such genetic modifications on xylose and glucose alcoholic fermentation.

Methods

Standard methods of yeast molecular genetics and biochemistry have been used.

Conclusions

Deletion of *CAT8* gene in *O. polymorpha* did not have effect on glucose alcoholic fermentation. At the same time, constructed knock out strains showed an improved xylose alcoholic fermentation relative to the parental strains. The ***cat8Δ*** strains isolated from the best available ethanol producer of *O. polymorpha* accumulated up to 12.5 g of ethanol/L at 45°C after 3 day fermentation without correction for evaporation or 25 times more relative to the wild-type strain. At the same time, overexpression of *CAT*8 inhibited xylose alcoholic fermentation. Accumulated data suggest that Cat8 protein acts as the specific regulator of xylose alcoholic fermentation. Expression of the genes involved in xylose fermentation was assayed.

FEMS7-0923
Biotechnology / Synthetic Biology / Systems Biology - Part III

UNCONVENTIONAL CATANIONIC MIXTURES OF GREEN SURFACTANTS: SYNERGIC ANTIMICROBIAL ACTIVITY

A. Ruiz[1,2], A. Pinazo[1], L. Pérez[1], A. Manresa[2], A. Marqués[2]
[1]IQAC-CSIC, Department of Chemical and Surfactant Technology, Barcelona, Spain
[2]Fac. of Pharmacy and Food Science - University of Barcelona, Lab. of Microbiology, Barcelona, Spain

Backgrounds

The huge consumption of surfactants in the biotechnological industry has created a strong demand for compounds that are more effective and environmentally friendly, as well as having new properties, such as antimicrobial activity. Catanionic mixtures present a wide variety of organized assemblies and aggregates with improved physicochemical and biological properties.

Objectives

In this work an anionic biosurfactant (lichenysin) was mixed with a battery of cationic amino acid-based surfactants ($C_3(CA)_2$, CAM, LAM, LLM, 1010R and $HisC_{12}$) in order to explore the surfactant molecular requirements for synergic antimicrobial activity.

Methods

The MICs of the surfactants and the catanionic mixtures were determined and the effect against the pathogens was observed using electron microscopy.

Conclusions

The gemini surfactant $C_3(CA)_2$ was found to be the most effective and its mixture was the only one that presented synergic antimicrobial activity against *Escherichia coli* O157:H7 (15.7 μM), *Yersinia enterocolitica* (7.8 μM), *Bacillus subtilis* (3.9 μM) and *Candida albicans* (7.8 μM). Electron microscopy images showed that $C_3(CA)_2$ changed its mode of action when mixed with lichenysin. Our hypothesis is that the catanionic aggregate is able to approach the anionic cell envelope by electrostatic interactions with its free cationic charge, which enables the lipopeptide to become attached to the cell membranes, thus enhancing the antimicrobial effect. On the other hand, when the cationic surfactant is completely neutralised, as occurred in the catanionic mixtures of lichenysin and monomeric amino acid-based surfactants, no synergic antimicrobial activity is detected.

FEMS7-2622
Biotechnology / Synthetic Biology / Systems Biology - Part III

EXTRACELLULAR ENZYMES PRODUCTION BY THE IMMOBILIZATION OF FILAMENTOUS FUNGI

Z. Suleimenova[1]
[1]*LLP Antigen, Biochemistry, Almaty, Kazakhstan*

Backgrounds

Almost all chemical reactions in a biological cell need enzymes which do differ from most other catalysts since they are highly specific for their substrates. As specific catalysts enzymes generally catalyze the conversion of only one type of substrate molecule into product molecules. That is why they are now finding widespread application in food and feed, beverage, detergent, biofuel, paper, brewing industries, molecular biology etc.

Objectives

Filamentous fungi are major producers of enzymes that have important applications in the food and beverage industries. The overall objective of this research is a strain improvement technology.

Methods

The new way of filamentous fungi cultivation method has been developed. It prolong producers' cultivation period up to 60 days and create the opportunity to obtain enzymes repeatedly in every 3 days of cultivation. This method is based on immobilizing enzymes producers with solid support in submerged conditions of growth. Design of proposed equipment gives the opportunity to increase the activity of immobilized cells culture filtrate comparing to free cells, growing in periodic culture conditions.

Conclusions

Such equipment construction allows to give a 10-times raise in fungal productivity; to prolong the process of fungi cultivation and periods of active culture liquid generation. Also, new devices and equipment give the way to improve quality of filtrates (to make them more clear) and exclude time-consuming processes.

FEMS7-2179
Biotechnology / Synthetic Biology / Systems Biology - Part III

ANTIBIOTICS PRODUCED BY PAENIBACILLI AND THEIR GENETICALLY MODIFIED DERIVATIVES AS A SOLUTION TO MULTIRESISTANT INFECTIONS

C. Salsano[1], S. Didelot[1], C. Barthélémy[1], V. Thiéry[1], R. Chevrot[1]
[1]Littoral Environnement et Sociétés LIENSs UMR CNRS 7266,
Institut du Littoral et de l'Environnement ILE Approches moléculaires environnement santé AMES, LA ROCHELLE, France

Backgrounds

With the rise in bacterial resistance and overuse of antibiotics, it is more and more difficult to find efficient treatments against human infections.

Objectives

One solution would be to discover new treatments or enhance existing molecules by genetic engineering.

Methods

In our team we have isolated two novel antibiotic producing strains of *Paenibacillus,* B-LR and P-32, from the environment and a sputum of a cystic fibrosis patient respectively. *Paenibacilli* are ubiquitous bacteria that produce antibiotics such as polymyxins and fusaricidins. These molecules are synthesized using specific enzymes called Nonribosomal Peptide Synthetases (NRPS). The strains B-LR and P-32 inhibit the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical strains (including methicillin resistant strains). These peptides were purified and characterized by LC-MS. B-LR produces polymyxin E (colistin) and ten different molecules while P-32 synthesizes a new depsipeptide. Two genomic libraries were constructed and screened in order to find the gene clusters responsible for the biosynthesis of these molecules. Thanks to this screening, the colistin gene cluster was discovered in B-LR. This new gene cluster covers 41 kb and includes 5 ORF. Three of them encode for NRPS involved in the colistin synthesis, whereas two others are ABC transporter-like genes that may release the antibiotic.

Conclusions

This study constitutes the first description of the biosynthesis pathway of this commercial antibiotic. Given that most of the antibiotics are nonribosomal peptides, our work could contribute to a better understanding of their biosynthesis. Moreover, it could lead to the biotechnological development of the pharmaceutical production of optimized antibacterial treatments.

FEMS7-2827
Biotechnology / Synthetic Biology / Systems Biology - Part III

CHARACTERIZATION OF A NOVEL ACETYLESTERASE AE-6L, USEFUL TO OBTAIN NEW FAMILIES OF LIPOPHILIC POLYPHENOLS WITH BROAD PHARMACOLOGICAL AND BIOTECHNOLOGICAL INTEREST

L. Sánchez Barrionuevo[1], A. González Benjumea[2], D. Cánovas López[3], J. Fernández Bolaños[2], I. Maya[2], Ó. López[4], M.T. García[1], E. Mellado[1]
[1]University of Seville, Department of Microbiology and Parasitology, Seville, Spain
[2]University of Seville, Department of Organic Chemistry, Seville, Spain
[3]University of Seville, Department of Genetics, Seville, Spain
[4]University of Seville, Department of Organic Chemistry-, Seville, Spain

Backgrounds

Natural phenolic compounds derived from olives display a potent and diverse profile of bioactivities, which make them excellent candidates for their use in pharmacology. However, the modification of this type of compounds to improve their properties is hindered by the limits of organic chemistry, as most of such procedures involve the use of hazardous reagents or non-green conditions, and they can only offer limited options.

Objectives

The aims of this work are to obtain a novel derivatives of the olive-derived phenolic compounds hydroxytyrosol, protocatechuic alcohol and 3,4-dihydroxyphenylglycol with improved lipophilic properties, but retaining their antioxidant capabilities

Methods

We have identified, cloned and purified an acetylesterase (AE-6L) from the lipolytic microorganism *Bacillus sp*. HR21-6, which can be used for tailoring a new set of compounds with broad biotechnological interest. The enzyme has been characterized including thermostability assays with the aim to optimize its industrial use. Site directed mutagenesis studies were performed to map the active sites and other conserved amino acids of AE-6L in order to identify the critical residues of the aminoacid sequence involved in the regioselectivity.
Finally, to substantiate the different bioactivities of these novel compounds, we have performed antioxidant, cytotoxicity and genotoxicity assays to evaluate their potential as anti-inflammatory compounds or in the prevention/treatment of cancer.

Conclusions

The performance of this work has allowed the development of novel catalytic procesess and the availability of a battery of semisynthetic regioisomers displaying different physicochemical properties.

FEMS7-1390
Biotechnology / Synthetic Biology / Systems Biology - Part III

ABRA1/A2, A KEY REGULATOR OF DEVELOPMENT AND ANTIBIOTIC PRODUCTION IN STREPTOMYCES COELICOLOR

R. Sánchez de la Nieta Moreno[1], *S. Antoraz Martín*[1], *R.I. Santamaría Sánchez*[1], *M. Díaz Martínez*[1]
[1]*University of Salamanca- CSIC, Department of Microbiology and Genetics, Salamanca, Spain*

Backgrounds

Two-component systems (TCSs) are included between the most important transduction signal mechanisms in bacteria, and are involved in many processes such as energetic metabolism or production of secondary metabolites. The AbrA1/A2 TCS has been described as a negative regulator of morphological differentiation and antibiotic production in *Streptomyces coelicolor*.

Objectives

The aim of this study is deciphering the regulon of AbrA1/A2 TCS.

Methods

Microarrays analysis comparing the wild-type strain and the corresponding mutant Δ*abrA1*/*A2* identified different expression of some key genes in metabolism (e.g. *SCO4562-4575*, *SCO4988-4990*), morphological differentiation (e.g. *chpA*, *rdlB*) and antibiotic production (e.g. *actII-4*, *redD*, *cdaR*). In this work, we use a promoter-probe system based in the production of the red antibioticic undecilprodigiosin to evaluate the regulation of promoters of interest *in vivo*. *S. coelicolor* M512 (a strain that does not produce any coloured antibiotic) and the isogenic mutant strain Δ*abrA1*/*A2* were used as hosts in the experiments.

Conclusions

The knowledge obtained may help us in the design of interesting strains to be applied in the biotechnology industry.

FEMS7-0977
Biotechnology / Synthetic Biology / Systems Biology - Part III

GENOME MINING OF THE LICHEN-ISOLATED STRAIN AMYCOLATOPSIS PRETORIENSIS CA-128772

M. Sánchez-Hidalgo[1], C. Díaz-Muñoz[1], I. González[1], J.R. Tormo[2], C. Moreno[2], J. Martín[2], O. Genilloud[1]
[1]Fundación MEDINA, Microbiology, Granada, Spain
[2]Fundación MEDINA, Chemistry, Granada, Spain

Backgrounds

Mining the genomes of microbial strains has become a main approach in the discovery of new secondary metabolites. While the genomes of *Streptomyces* spp. have been widely explored, *Amycolatopsis* spp. genomes have begun to be sequenced recently[1]. Strains from this genus are known to produce commercially important antibiotics such as balhimycin, vancomycin or rifamycin, which biosynthetic gene clusters (BGCs) have been characterized[2]. Many other cryptic BGCs have also been identified in the genomes of these strains, which pave the way for the discovery of novel compounds.

Objectives

Our main objective is to mine the genome of *Amycolatopsis pretoriensis* CA-128772*,* isolated from lichen samples collected in tropical areas from Hawaii[3]*,* in order to identify and characterize cryptic BGCs.

Methods

The *A. pretoriensis* CA-128772 genome was analyzed with antiSMASH[4] and PRISM[5] to detect potential BGCs. The strain was fermented in different media and incubation times. Fermentation broths were extracted and the metabolite profile of the extracts was analyzed.

Conclusions

The draft genome size was around 10.2 Mb with a GC content of 71.8%. A total of 140 putative BGCs were predicted, including lanthipeptides, lassopeptides, PKS-I, NRPS, terpenes, siderophores and bacteriocins. The connection between the predicted pathways and the detected compounds was established.

References

1. Rashmi *et al.* (2016) Indian J. Microbiol. 56: 233-246
2. Chen *et al.* (2016) ChemBioChem 17: 119-128
3. González *et al.* (2005) FEMS Microbiol. Ecol. 54: 401-415
4. Weber *et al.* (2015) Nucl. Acids Res. 43: W237-W243
5. Skinnider *et al.* (2015) Nucl. Acids Res. 43: 9645-9662

FEMS7-2689
Biotechnology / Synthetic Biology / Systems Biology - Part III

THE FUSOGENIC PEPTIDE HA2 FROM INFLUENZA VIRUS IMPACTS ON THE CELL SELECTIVITY OF CXCR4-TARGETED PROTEIN NANOPARTICLES

L. Sánchez[1], N. Serna[1], M. Mattanovich[2], P. Cazzanelli[2], A. Sánchez[3], F. Cortés[4], U. Unzueta[5], R. Mangues[5], A. Villaverde[1], E. Vázquez[1]
[1]Institut de Biotecnologia i Biomedicina Universitat Autonoma de Barcelona, Departament de Genetica i Microbiologia, Barcelona, Spain
[2]University of Natural Resources and Life Sciences, Department of Biotechnology, Vienna, Austria
[3]Universitat Autonoma de Barcelona, Servei de Microscopia, Barcelona, Spain
[4]Universitat Autonoma de Barcelona, Servei de Cultius Cel·lulars- Producció d'Anticossos i Citometria SCAC, Barcelona, Spain
[5]Hospital de la Santa Creu i Sant Pau, Institut d'Investigacions Biomèdiques Sant Pau, Barcelona, Spain

Backgrounds

We have recently described an architectonic principle at the nanoscale that allows generating self-assembling nanoparticles formed by engineered proteins produced in bacteria. T22 is a cationic ligand of the cell surface cytokine receptor CXCR4, that specifically binding CXCR4+ cells also acts as an architectonic tag in GFP-based particles (T22-GFP-H6). Since CXCR4 is overexpressed in diverse malignant cancers, including colorectal cancer, pancreatic cancer and lymphoma, these protein constructs are promising vehicles for targeted drug delivery.

Objectives

The aim of this project was to insert the fusogenic peptide hemagglutinin-2 (HA2) from influenza virus to the protein T22-GFP-H6 in order to evaluate potential enhancement of cell internalization of the modified nanoparticles. HA2 is found on the surface of virus particles and is known to destabilize the lipid cell membrane to promote escape from the endosomes at acidic pH.

Methods

The engineered proteins were produced in *Escherichia coli* and purified through a His tag affinity chromatography. Physicochemical characterization was performed using Coomassie blue staining, Western blot, mass spectrometry, Dynamic Light Scattering and confocal and scanning electron microscopy. Cultured CXCR4+ Hela cells were also used for *in vitro* experiments to elucidate the internalization kinetics.

Conclusions

The addition of HA2 in two different positions (T22-HA2-GFP-H6 and T22-GFP-HA2-H6) provides an enhanced cell penetration although it is accompanied by a loss of specificity, especially when the HA2 is placed close to T22. Probably it is due to intra or intermolecular interactions that affect the exposure of the ligand, which is critical for the nanoparticle’s internalization.

FEMS7-2036
Biotechnology / Synthetic Biology / Systems Biology - Part III

EXTERNALLY ADDED CHOLINE-BINDING MODULES (CBMS) TARGET THE PNEUMOCOCCAL CELL WALL AND AFFECT BACTERIAL GROWTH

M. Sánchez[1], B. Maestro[2], E. Roig[2], F. Garcia-Asencio[3], J. Sanz[2]
[1]Universidad Miguel Hernandez, Dpt. Vegetal Production and Microbiology, Elche - Alicante, Spain
[2]Universidad Miguel Hernandez, Instituto de Biología Molecular y Celular, Elche - Alicante, Spain
[3]Universidad Miguel Hernandez, Instituto de Neurociencias, San Juan de Alicante - Alicante, Spain

Backgrounds

Streptococcus pneumoniae (pneumococcus) is one of the major bacterial pathogens. Due to the raise of antibiotic resistance, the search and development of new antimicrobial molecules is one of the main needs in modern Public Health services.

Objectives

One of the most attractive targets are the choline-binding proteins (CBPs). These are essential for cell processes such as viability, host colonization and virulence. Pneumococcal CBPs make use of the so-called choline-binding modules (CBMs) to specifically adsorb onto the bacterial cell wall through binding to the phosphorylcholine residues in the teichoic acids. In vitro experiments had shown that addition of CBMs from the LytA amidase, CbpD protein and the phagic CPL1 lysozyme (C–LytA, C-CbpD and C–CPL1 respectively) competes with their parental cell-wall hydrolases and inhibit their function. Thus CBMs might constitute a novel kind of anti-pneumococcal molecules.

Methods

To evaluate that, we first estimated the binding affinities of C–LytA, C-CbpD and C–CPL1 to DEAE-functionalised magnetic nanoparticles as bio-inspired mimics of the cell wall. The results indicate that all modules greatly differ in their binding mode and affinity to both free choline and DEAE nanoparticles.

Next, all CBMs were externally added to exponential cultures of *Streptococcus pneumoniae* R6. The chimeric proteins bind to the bacterial surface immediately and caused the inhibition of cell separation, leading to long bacterial chains and sedimenting aggregates.**Conclusions**

This results suggests that the use of CBMs might represent promising candidates for a streamlined action against pneumococcus based on bacterial aggregation and subsequent phagocytosis.

FEMS7-1854
Biotechnology / Synthetic Biology / Systems Biology - Part III

GROWTH OF THERMUS THERMOPHILUS IN WATER-IN-OIL MICRODROPLETS

M. Sanchez[1], *E. Sanchez*[1], *J. Berenguer*[1], *A. Hidalgo*[1]
[1]*Centro de Biología Molecular Severo Ochoa UAM-CSIC, MOLECULAR BIOLOGY, MADRID, Spain*

Backgrounds

Microfluidic devices allow the generation of monodisperse water in oil microdroplets of picoliter volume that can be subsequently used for the incubation and manipulation steps required for super-high throughput screening at very low cost in personnel and equipment. The inclusion of single cells into these microdroplets and their growth could be used for in vivo screening of enzymes and products as also for cell biology studies

Objectives

To develop protocols for the growth of extreme thermophiles in water in oil microdroplets

Methods

Thermus thermophilus derivatives expressing thermostable derivatives of the Yellow Fluorescent Protein were included in microdroplets to analyse their growth capability by fluorescent microscopy

Conclusions

T. thermophilus can growth for several hours and form microcolonies inside microdroplets. Such microdroplets are stable for days and can be subjected to enzyme-presence assays

This work has been funded by the European project “MetaFluidics” (GA nº 685474)

FEMS7-1354
Biotechnology / Synthetic Biology / Systems Biology - Part III

SELECTION AND IDENTIFICATION OF CHITINASE PRODUCING BACTERIA RECOVERED FROM THE ANTARCTIC ENVIRONMENT

Y. Santa Cruz Vasquez[1], *A. Duarte Fernandes*[2], *T. Rodrigues e Silva*[1], *V. Maia de Oliveira*[1]
[1]*University of Campinas UNICAMP, Institute of Biology, Campinas, Brazil*
[2]*Federal University of Alagoas, Campus Arapiraca, Arapiraca, Brazil*

Backgrounds

The Antarctic environment has extreme climatic conditions, especially low temperatures, high incidence of UV radiation and water deficit. In this sense, microorganisms that survive in this environment are known as psychrophiles and psychrotolerant or "cold tolerant". So, the isolation of microorganisms may allow access to a genetic resource with biotechnological potential and little explored.

Objectives

The aim of this study was to investigate the ability of bacterial isolates recovered from Antarctica to produce cold-active chitinase at low temperature.

Methods

A total of 560 bacteria were isolated from marine sediments, macroalgae, sea star and sea sponge samples collected in Antarctica during the summer of 2013 following cultivation in R2A medium and incubation at 15 ºC. The functional screening was performed using c*olloidal chitin prepared* from shell of crab (Sigma) as carbon source. These assays were evaluated in 96-deep well plates incubated at 15 ºC and all positives strains were cultured individually at 5 and 15 °C during 326 h. Identification of the chitinase producers was done based on sequencing and phylogenetic analysis of 16S rRNA gene using *10f and 1100r* primers.

Conclusions

Nineteen chitinase producing bacteria producers of chitinase were identified as belonging to the genus Arthrobacter (A. psychrochitiniphilus and A. alpinus) and one to the genus Curtobacterium (C. luteum). The largest halos indicatingchitinase production were observed infor A. psychrochitiniphilus 492, followed by A.psychrochitiniphilus 363 of under incubation at 15 °C for 326 h. These bacteria are promising candidates for biotechnological processes at low temperature.

FEMS7-2573
Biotechnology / Synthetic Biology / Systems Biology - Part III

TAXONOMIC ASSESSMENT AND ANTIMICROBIAL SCREENING OF BACTERIA ISOLATED FROM MARINE AND TERRESTRIAL ANTARCTIC SAMPLES

Y. Santa Cruz[1], T. Rodrigues e Silva[1], A. Fernandes Duarte[2], V. Maia de Oliveira[1], F. Fantinatti-Garboginni1[1], M. Zambrano Passarini[3]
[1]University of Campinas, Institute of Biology, Campinas, Brazil
[2]Federal University of Alagoas, Campus Arapiraca, Arapiraca, Brazil
[3]Latin-American University UNILA, Biotechnology, Foz do Iguaçu, Brazil

Backgrounds

Microorganisms dominate most of the Antarctic environment and play a crucial role in its functioning and primary productivity. Several numbers of studies have shown that there is a great biodiversity in the polar regions and different types of biological activities.

Objectives

The aim of the present study was to survey microorganisms from Antarctic samples in the search for antimicrobial and antagonistic effects of clinical interest.

Methods

The screening of antimicrobial substances was performed by the agar diffusion method. Six hundred strains previously isolated from Antarctic samples were tested against the indicator strains *E. coli, Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis* and *Candida albicans* for antimicrobial production. Bacterial identification was achieved by comparing the 16S rRNA sequences obtained with sequence data from reference type strains, as well as public databases GenBank and RDP.

Conclusions

Phylogenetic analysis revealed that most isolates shared high sequence similarity to recognized species, including those recovered previously from the Antarctica environment, which belong to four major phyla: Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (Alpha and Gammaproteobacteria classes). A total of 361 bacterial strains, distributed in 38 different genera, were identified based on the 16S RNAr gene. The major representatives were *Arthrobacter*, *Psychrobacter*, *Pseudoalteomonas* and *Rhodococcus*. Antimicrobial screening revealed sixteen strains capable of inhibiting growth of, at least, one of the indicator strains. The psychrotolerant bacterial isolate *Pseudomonas mandelii* 99 showed a broad antimicrobial spectrum and low values in MIC assay and was then selected for further antiproliferative and antiparasitic tests. Our findings demonstrated that the extremophilic bacteria from Antarctica represent an untapped source of microorganisms capable of antimicrobial metabolite production.

FEMS7-1310
Biotechnology / Synthetic Biology / Systems Biology - Part III

UNDERSTANDING THE RATE LIMITING STEP IN XYLOSE UPTAKE IN CRYPTOCCOCUS HUMICOLA FOR BIOETHANOL PRODUCTION

A.C. Ramos Validvia[1], T. Ponce Noyola[1], L.M. Salgado Rodriguez[2], A. Santiago Benítez[1]
[1]CINVESTAV, Biotechnology and Bioengineering, Mexico City, Mexico
[2]CICATA, Scientific and Technologic Research, Queretaro, Mexico

Backgrounds

The fermentation of hexoses and pentoses of lignocellulosic origin is sought in the process of bioethanol production. Xylose is the second most abundant sugar in agroindustrial residues; for this reason scientists have searched microorganisms capable of xylose assimilation and fermentation. *Cryptococcus humicola* with the ability to grow in xylose, was isolated from *mezcal* mash in Oaxaca, Mexico. Due to its ability to also grow and ferment glucose and cellobiose, it is proposed as a good object of study for possible industrial application. Xylose reductase (XR) and xylitol dehydrogenase (XDH) are key enzymes in yeast for the production of ethanol from xylose.

Objectives

To understand the rate limiting step in xylose fermentation in *C. humicola* through the study of the enzymatic activity of XR and XDH, in addition to their level of expression.

Methods

C. humicola was cultivated in minimum medium in order to observe the inductive effect of the carbon source and aeration on enzyme activity. XR and XDH specific activities were measured under these conditions.

Conclusions

It was observed that even when the highest values of specific activity were found when the yeast was grown in xylose and in anaerobic conditions, xylose consumption and xylitol production were lower compared to aerobic conditions. It is, therefore, proposed that xylose intake seems not entirely dependent of enzymatic activity of XR and XDH or enzyme quantity but it is rather limited by other factors like redox state imbalance or low enzyme activity further in the route.

FEMS7-1713
Biotechnology / Synthetic Biology / Systems Biology - Part III

DESIGNING RECOMBINANT BIOCATALYSTS FOR THE EFFICIENT REMOVAL OF AROMATIC ENDOCRINE DISRUPTING COMPOUNDS

D. Sanz[1], *J.L. García*[1], *E. Díaz*[1]
[1]*CIB-CSIC, ENVIRONMENTAL BIOLOGY, Madrid, Spain*

Backgrounds

Endocrine disruptors chemicals (EDCs) are persistent contaminants that distrub the endocrine system of humans and animals. The alkyl or di-alkyl esters of *o*-phthalic acid (1,2-benzenedicarboxylic acid) are synthesized in large quantities for the plastic industry and constitute one of the main sources of EDCs with carcinogenic activities [1].

Despite microbial degradation is an ecosystem friendly strategy to remove phthalates only few microorganisms have been identified as phthalates degraders in aerobic conditions and none of them degrade anaerobically phthalate esters. Hence, improving our knowledge on the biodegradation of toxic phthalates is crucial to develop new detoxification strategies.

Objectives

Engineering recombinant biocatalyst for the efficient removal of phthalates both under aerobic and anaerobic conditions.

Methods

Construction of mobilizable synthetic catabolic cassettes containing the genes for the uptake and conversion of *o*-phthalate to the central intermediate benzoyl-CoA [2], Efficient expression of the cassetes in model bacteria of the *Azoarcus* and *Cupriavidus* genus able to degrade benzoyl-CoA under anaerobic and/or aerobic conditions. Improving *o*-phthalate cassettes by engineering phthalate esterases able to remove the alkyl side chain(s) of the EDC phthalates.

Conclusions

Recombinant bacterial strains have been engineered for the first time for the efficient uptake and removal of *o*-phthalate and its mono- and di-alkyl esters under anaerobic (*Azoarcus* strains) or aerobic (*Azoarcus* and *Cupriavidus* strains) conditions. This work paves the way for a promising alternative to current phthalates detoxification strategies.

1. *Environ. Int.* **76,** 78–97 (2015).
2. *et al. The ISME Journal* (2017) **11**, 224–236

This work has been funded by Ramón Areces foundation.

FEMS7-1700
Biotechnology / Synthetic Biology / Systems Biology - Part III

BIOENERGETICS OF PHOTOFERMENTATTION: EFFECT OF PROTONOPHORES OF MEMBRANE-ASSOCIATED ATPASE ACTIVITY IN RHODOBACTER SPHAEROIDES

H. Sargsyan[1], L. Gabrielyan[1], A. Trchounian[1]
[1]Yerevan State University, Biochemistry- Microbiology and Biotechnology, Yerevan, Armenia

Backgrounds

Nowadays, the current interest of bioenergetics in purple bacteria is their hydrogen (H_2) production ability. This process is mediated by nitrogenase, which requires energy of ATP; proton motive force is also required.

Objectives

In this work the effects of two protonophores (carbonyl cyanide m-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNP)), and *N,N'*-dicyclohexylcarbodiimide (DCCD) on the F_oF_1-ATPase activity in *Rhodobacter sphaeroides* MDC6521 (from Armenian mineral springs) were studied in various illumination conditions.

Methods

The bacterium was cultivated in Ormerod medium anaerobically with carbon source – succinate. ATPase activity was measured by amount of inorganic phosphate, liberated after adding ATP to membrane vesicles of *R. sphaeroides,* which were prepared by Kaback's method. The H_2 yield was evaluated by the redox potential drop to low negative values during bacterial growth.

Conclusions

CCCP and DNP dissipate the proton motive force, which is responsible for ATP synthesis, whereas DCCD inhibits the ATP synthesis by blocking the proton flux through the F_oF_1-ATPase. The F_oF_1-ATPase activity of membrane vesicles was inhibited by DCCD on ~2 fold. Both protonophores stimulate ATPase activity on ~1.5-fold, which might be connected with compensation of proton gradient, dissipated by protonophores. H_2 yield is decreased ~5.0-7.5-fold in the presence of 0.5 mM CCCP and 10 mM DNP, respectively; and is not observed by addition of 2 mM CCCP and 50 mM DNP. This effect is coupled with suppression of ATP synthesis by photophosphorylation, which is important for nitrogenase-dependent photofermentative H_2 production by purple bacteria.

The results obtained would be important for understanding of bioenergetics of photofermentative H_2 production.

FEMS7-1259
Biotechnology / Synthetic Biology / Systems Biology - Part III

MODULATING THE REDOX HOUSEHOLD OF LACTOBACILLUS DIOLIVORANS BY THE CARBON FEED

A.K. Lindlbauer[1], H. Marx[1], M. Sauer[2]
[1]BOKU - University of Natural Resources and Life Sciences, Department of Biotechnology, Vienna, Austria
[2]BOKU - University of Natural Resources and Life Sciences, Department of Biotechnolog, Vienna, Austria

Backgrounds

Biorefineries can only be economically viable, when no waste stream is created, but the harvested biomass is entirely used and valorized. Biodiesel biorefineries produce large amounts of lignocellulosic biomass remaining from oil milling and the transesterification process leads to crude glycerol as a major side stream. Microbial processes for the valorization of these side streams to fuels or chemicals are highly desirable. It appears crucial for those tasks to exploit nature's biodiversity. Here, we report about an economic process for 1,3-propanediol production with the lactic acid bacterium *Lactobacillus diolivorans*. While the organism cannot grow on glycerol as sole carbon source it readily uses glycerol as electron acceptor, when a sugar is present.

Objectives

The objective of this work was to characterize the metabolism of *L. diolivorans* in respect to the carbon feeding strategy. Glucose and glycerol are co-metabolized. Part of the glycerol is reduced to 1,3-propanediol, while another part is oxidized to 3-hydroxypropanoic acid.

Methods

The microorganisms are grown in bioreactors under tightly controlled conditions. Different carbon feeding strategies have been tested and the cultures have been analyzed in respect to carbon uptake and metabolite accumulation.

Conclusions

Concluding, we can show that a pulsed addition of glucose shifts the glycerol metabolism nearly exclusively to its reductive branch, yielding 1,3-propanediol. This is in contrast to a constant glucose feed, which leads to the oxidation of a significant fraction of the glycerol. We speculate that not the uptake rate but the concentration of glucose in the medium is the decisive factor for the redox metabolism.

FEMS7-2864
Biotechnology / Synthetic Biology / Systems Biology - Part III

SCALING UP BIOREMEDIATION IN SOILS HIGHLY POLLUTED WITH HYDROCARBONS

M. Seeger[1], R. Orellana[2], M. Gonzalez[3], F. Cárdenas[3], A. Cumsille[3], C. Rojas[2], C. Stuardo[3], R. Wittwer[3], E. Becerra[3]
[1]Universidad Tecnica Federico Santa Maria, Chemistry, Valparaiso, Chile
[2]Universidad Técnica Federico Santa María, Chemistry, Valparaíso, Chile
[3]Universidad Técnica Federico Santa María, Chemistry, Valparaiso, Chile

Backgrounds

Petroleum hydrocarbon pollution is a global problem faced within the vicinity of urban and industrial areas and represent a significant health risk. Microbial degradation of hydrocarbons have been applied for bioremediation of polluted soils. Bioremediation approaches are often based on bioaugmentation using exotic hydrocarbon-degrading microorganisms or on biostimulation of the native communities using nutrients. The main knowledge on microbial degradation is based on lab scale studies, whereas its variation under *in situ* conditions remains unknown.

Objectives

The aim of this study is to compare bioremediation rates in hydrocarbon polluted soils at lab scale and field scale experiments.

Methods

On one side, bioaugmentation and biostimulation incubation experiments based on 1 L mesocosms were carried out. On the other side, degradation kinetic to similar treatments of 400 L biopiles were carried out outdoor during two months. For bioaugmentation a consortia of selected bacterial strains were applied. Biostimulation was done by the addition of organic amendments. Hydrocarbons were quantified by GC-FID. Culturable heterotrophic bacteria were monitored by CFU counting.

Conclusions

Both bioremediation approaches were able to remove hydrocarbons from soils at lab and field scale. At similar initial concentration of petroleum hydrocarbons, higher degradation rates in lab scale experiments were observed. In conclusion, laboratory scale studies, where conditions are acutely controlled and microbial communities can be managed, yielded higher degradation rates. Key factors determining the difference in degradation rates are discussed. This study emphasizes the need for more detailed analysis in field studies, which are crucial to move on industrial bioremediation of soils polluted with hydrocarbons.

FEMS7-1062
Biotechnology / Synthetic Biology / Systems Biology - Part III

METABOLIC ENGINEERING OF THE YEAST SACCHAROMYCES CEREVISIAE FOR CONSTRUCTION OF ANAEROBIC OVERPRODUCER OF GLYCEROL

M. Semkiv[1], K. Dmytruk[1], C. Abbas[2], A. Sibirny[1]
[1]Institute of Cell Biology NAS of Ukraine, Department of Molecular Genetics and Biotechnology, Lviv, Ukraine
[2]Archer Daniels Midland Co, Research Center, Decatur IL, USA

Backgrounds

Glycerol is widely used in the cosmetic, food and pharmaceutical industries. The promising way for glycerol production is microbial fermentation. Yeast *Saccharomyces cerevisiae* could be used for cost-effective glycerol production under anaerobic conditions, however, such producers have not been described yet. In *S. cerevisiae* glycerol synthesis starts from dihydroxyacetone phosphate by subsequent action of glycerol-3-phosphate dehydrogenase (Gpd1) and glycerol-3-phosphate phosphatase (Gpp2). Synthesized glycerol is exported via channel formed by aquaglyceroporin Fps1. In the wild-type strains, dihydroxyacetone phosphate is predominantly isomerized to glyceraldehyde-3-phosphate by triose phosphate isomerase (Tpi1) and subsequently converted to pyruvate and eventually to ethanol. Enzyme acetolactate synthase (Ilv2) can convert pyruvate to acetolactate and CO_2 thus decreasing amount of pyruvate available for alcohol dehydrogenase reaction which competes for NADH with Gpd-reaction.

Objectives

We aimed to construct recombinant strain with reduced Tpi1 activity, increased Gpd1 and Gpp2 activities, constantly active form of Fps1 channel and expression of cytosolic form of Ilv2.

Methods

Homologous recombination was used for partial substitution of *TPI1* gene promoter region with selective marker. Multicopy integration module was used for expression of hybrid fused *GPD1-GPP2* ORF under the control of strong promoter. Modified gene *FPS1* (with eliminated part encoding amino acid residues 76-230) and truncated gene *ILV2* were expressed under the control of *ADH1* promoter.

Conclusions

The constructed strain BY/tpi25/gpd1gpp2f/fps1m/ilv2 accumulated up to 9 times more glycerol under micro-aerobic conditions and up to 4.7 times more glycerol under anaerobic conditions relative to the parental strain. The increase in glycerol production led to a drop in ethanol and biomass accumulation.

FEMS7-2335
Biotechnology / Synthetic Biology / Systems Biology - Part III

ANTIBIOTIC-FREE SELECTION PLASMIDS IN STREPTOMYCES

L. Sevillano[1], M. Diaz[1], R.I. Santamaria[1]
[1]Salamanca University, IBFG, SALAMANCA, Spain

Backgrounds

Streptomyces is a promising bacterial expression system that has been used to produce high levels of several proteins. In previous work, we described a new strategy for the stabilization of expression plasmids in *Streptomyces* based on the use of a toxin-antitoxin system (*yefM/yoeBsl*) as selection marker. We demonstrated the effectiveness of this system for the production of high levels of proteins without the use of antibiotics during the production step. However, the host used and the expression vectors still have the antibiotics resistance genes needed for its construction.

Objectives

The objective of our work is the improvement of this system by the elimination of the antibiotic resistance genes still present in the host and in the expression plasmid.

Methods

We have integrated the toxin gene (*yoeBsl*) in the genome with a new plasmid, pTES-tox, that has the target sites for the Cre recombinase (*loxP*) flanking the toxin gene and the phage attachment site (*attP*). Thus, after the expression of Cre recombinase we have deleted the plasmid backbone leaving only the toxin gene in the genome. In the same way, we have introduced the target sites for the Dre recombinase (*rox*) flanking the neomycin gene in the expression plasmid, the subsequent expression of the recombinase allowed us to delete it.

Conclusions

We describe a system completely free of antibiotic resistance genes that is useful for the production of high yields of proteins in *Streptomyces*. This system could be a powerful tool for using *Streptomyces* as host to produce proteins at industrial and pharmaceutical level.

FEMS7-0317
Biotechnology / Synthetic Biology / Systems Biology - Part III

HIGH CAROTENOID PRODUCTION BY A HALOTOLERANT BACTERIUM, KOCURIA SP. STRAIN QWT-12 AND ANTICANCER ACTIVITY OF ITS CAROTENOID

S.A. Shahzadeh fazeli[1], A. Safarpour[2], M.A. Amoozegar[3]
[1]Iranian Biological Resource Centre, Microorganisms bank, Tehran, Iran
[2]University of Science and Culture, 4. Department of Developmental Biology, tehran, Iran
[3]University of Tehran, Department of Microbiology, Tehran, Iran

Backgrounds

Carotenoids are yellow to orange-red pigments widely distributed among nature of many organisms. Their existence happens from bacteria, yeast and algae to animal and plant cells

Objectives

Halophilic prokaryotes are extremophile microorganisms that grow optimally in media containing salts and almost appeared pigmented and many of them contain high concentrations of carotenoids. Several halophiles isolated from industrial wastewaters with upper mediate salinity.

Methods

Water samples were obtained from tannery wastewater of a company in Qom, Iran. After cultivation of samples, yellow colonies were selected and purified. Carotenoids of the selected strain were extracted by methanol. MTT assay for extracted Carotenoid was carried out to assess the viability of seven cancer cell lines belonging to Breast, Lung and Prostate cancer with negative control of fibroblast cell line through six concentration levels to find out IC50. .

Conclusions

Strain QWT-12 is a Gram-stain-positive coccoid, aerobic, non-endospore-forming, halotolerant bacterium and showed high capacities in the production of yellow carotenoids in a wide range of culture medium factors. Based on statistical analysis of data from MTT assay, IC50 of 1, 4, 4 and 8 mg/ml for MCF-7, A549, MDA-MB-468 and MDA-MB-231 was found respectively. According to the obtained results from TLC and Mass Spectrophotometry, absorption spectrum of carotenoid from strain QWT-12 is similar to the absorption spectrum of the carotenoid neurosporene. In this study we exhibited that strain QWT-12 has pigment likewise nourosporene pigments as major carotenoid pigment and its pigment were found to be cytotoxic against several cancer cell lines after 48 h with cell line specific activities. Carotenoids have been shown to suppress the cancer cell *in vitro* and *in vivo* and among different carotenoids,

FEMS7-0946
Biotechnology / Synthetic Biology / Systems Biology - Part III

CAROTENOIDS PRODUCTION WITH BIOLOGICAL ACTIVITY BY RHODOTORULA MUCILAGINOSA FROM SOLID WASTE COFFEE PROCESSING

C. Silva[1], M. Dias[1], M. Melo[1], J. Coimbra[1], R. Schwan[1], L. Batista[2]
[1]Federal University of Lavras, Biology/ Microbiology, Lavras, Brazil
[2]Federal University of Lavras, Food Science, Lavras, Brazil

Backgrounds

The three coffee process generates liquid and solid waste known as husks, pulp and waterwaste. Specially husks and pulp can be used as substrate to fermentation process producing add-value compounds.

Objectives

This work was aimed to evaluate the antioxidant and antimicrobial activities of carotenoids produced by *Rhodotorula mucilaginosa* CCMA 0156 in submerged fermentation using coffee processing solid waste

Methods

The production medium contained coffee pulp extract (6.68 %) added by yeast extract (3 g/L), peptone (10.04 g/L), glucose (2 g/L) and Tween 80 (0.5 %) or husk extract (8.36 %) added by peptone (3.68 g/L), glucose (6.36 g/L) and Tween 80 (0.5 %). The production of total carotenoids by yeast in the pulp extract was 16.36 mg/L (being 4.1% compound of β-carotene) and husk extract was 21.35 mg/L (being 4.5% compound of β-carotene).

Conclusions

Carotenoids produced, exhibited antioxidant and antimicrobial activities against pathogenic bacteria as *Salmonella colorless*, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* and toxigenic fungi as *Aspergillus flavus*, *A. parasiticus*, *A. carbonarius* and *A. ochraceus.* These characteristics of these pigments are important for many industries. Mainly the food industry has become increasingly interested in the use of microbial technology to produce colors for use in foods. It can also help to overcome the growing public concern over the adverse health effects of addition of synthetic colors in food products.

Financial support: CAPES, CNPq, FAPEMIG

FEMS7-1423
Biotechnology / Synthetic Biology / Systems Biology - Part III

BIOEMULSIFIER ACTIVITY ANALYSIS OF NOVEL PROTEIN FROM BACILLUS SUBTILIS

A. Simancas[1], T. Weiyi[2], F. Molina[1], S. Li[2]
[1]Universidad de Extremadura, Bioquímica- Biología Molecular y Genética, Badajoz, Spain
[2]Nanjing Tech University, School of Biotechnology and Pharmaceutical Engineering, Nanjing, China

Backgrounds

Biofilms in dairy industry are produced in instruments by contaminant bacteria from genus such as *Bacillus* and *Staphylococcus*, among others. These structures confer increased bacterial resistance to antibiotics and both chemical and mechanical removal, leading to a higher risk of microbiological contamination during the manufacturing processes. Despite being extensively used; these methods are often not efficient. Thus, we have started to develop a biocontrol strategy based on the use of bioemulsifier proteins, with the final aim of creating a bacterial strain suitable for reducing or eliminating these biofilms.

Objectives

We wanted to test *in vitro* bioemulsifier activity as well as emulsion stability of our novel isolated protein from *Bacillus subtilis* (strain CICC 20034), still under characterization, using different kinds of substrates, media and conditions. Additionally, we have compared the protein activity with other detergents such as SDS, Tween 20 or household detergent.

Methods

Novel protein was isolated and purified through HPLC techniques. Bioemulsifier activity was assayed, along with other detergents, measuring emulsion layer on glass vials with a 1:1 proportion of sterile water and substrate after 2 days of incubation.

Conclusions

The protein we have studied has proved in our *in vitro* tests to be a reliable alternative to the use of other detergents, leading to a possible application in the removal of biofilms in the dairy industry.

FEMS7-2983
Biotechnology / Synthetic Biology / Systems Biology - Part III

EFFECT OF TRIMETHYLATED POLY-(D)GLUCOSAMINE NANOPARTICLES CARRYING BACTERIOFERRITIN PROTEIN OF HELICOBACTER PYLORI FOR CANCER TREATMENT

N. Soleimani[1]
[1]*Shahid Beheshti University- Faculty of Life Sciences and Biotechnology, Department of Microbiology and Microbial Biotechnology, tehran, Iran*

Backgrounds

Breast cancer is one of the leading causes of death in women around the world. Conventional treatments use cytotoxic drugs which have high levels of side effects. Today's pharmacology is looking novel drugs for treatments with low side effects and maximum efficiency. The Helicobacter pylori neutrophil-activating protein (bacterioferritin) is a virulence factor that attracts and activates neutrophils, and promotes their endothelial adhesion and the production of oxygen radicals and chemokines. bacterioferritin is an immune modulator able to induce the expression of IL-12 and IL-23. In this study, we were evaluate, Preparation of trimethylated Poly-(D)glucosamine Nanoparticles carrying recombinant bacterioferritin Helicobacter pylori pylori for Breast cancer treatment.

Objectives

In this study, we were evaluate, Preparation of trimethylated Poly-(D)glucosamine Nanoparticles carrying recombinant bacterioferritin Helicobacter pylori pylori for Breast cancer treatment.

Methods

In this study, purification of recombinant H. pylori bacterioferritin was performed by Ni–NTA affinity chromatography. trimethylated Poly-(D)glucosamine Nanoparticles were produce. The size and morphology of the nanoparticles were investigated. recombinant bacterioferritin Helicobacter pylori were produce. SDS-PAGE analysis showed the expression of an approximately 20000 Dalton protein. Statistical analysis showed that bacterioferritin had not toxic effects on 4T1 cells in a concentration of 500 ng/ml after 24 and 48 h, and this toxic dose was not after 72 h.

Conclusions

bacterioferritin had not direct toxic effects on Breast cancer cell and the toxicity was observed when in vivo study. Perhaps This protein can active T lymphocyte and this cell secretory effective cytokine and damage cancer cell. The complex has the potential to shift antigen-specific T-cell responses from a predominant Th2 to a polarized Th1 cytotoxic phenotype, characterized by high levels of interferon-γ and tumor necrosis factor-a production. bacterioferritin may be a new tool for future therapeutic strategies aimed in cancer immunotherapy.

FEMS7-0491
Biotechnology / Synthetic Biology / Systems Biology - Part III

PRODUCTION OF BERRY ANTHOCYANINS IN LACTOCOCCUS LACTIS

A. Solopova[1], P. Gaspar[2], J. Förster[2], O.P. Kuipers[1]
[1]GBB, Molecular Genetics, Groningen, The Netherlands
[2]Danmarks Tekniske Universitet, Biosustain, Hørsholm, Denmark

Backgrounds

Anthocyanins have been used in folk medicine for generations, but only recently the specific pharmacological properties of these compounds became the target of extensive studies. In addition to their antioxidant properties, anthocyanins are valuable for their colors. The food industry actively searches for natural alternatives, such as anthocyanins, to replace synthetic dyes. Exploitation of microbial hosts as cell factories is an attractive alternative to extraction of anthocyanins and other flavonoids from plant sources or chemical synthesis. Lactic acid bacterium *Lactococcus lactis* is a promising host for production of plant high value chemicals. It has a long history of safe usage in food fermentations and has been granted a GRAS (Generally Regarded As Safe) status.

Objectives

Our aim was to reconstruct the pathway for plant anthocyanin cyanidin 3-*O*-glucoside production from flavan-3-ol catechin in a Gram-positive bacterium *L. lactis*.

Methods

Native genes coding for anthocyanidin synthase and anthocyanidin 3-*O*-glucosyltransferase from plants and synthetic codon-optimized genes were assembled to form a functional metabolic pathway in *L. lactis*.

Conclusions

Plant enzymes anthocyanidin synthase and anthocyanidin 3-*O*-glucosyltransferase were codon optimized and expressed in *L. lactis.* Anthocyanidin cyanidin and anthocyanin chrysanthemin (cyanidin 3-*O*-glucoside) were produced from flavan-3-ol catechin by engineered *L. lactis* strains and accumulated intracellularly. In summary, lactic acid bacterium *L. lactis* appears to be a valuable production host for plant-derived bioactive compounds for food applications.

FEMS7-2725
Biotechnology / Synthetic Biology / Systems Biology - Part III

SOXR AS A SINGLE-CELL BIOSENSOR FOR OPTIMIZING NADPH-CONSUMING ENZYMES IN ESCHERICHIA COLI

A. Spielmann[1], *S. Siedler*[2], *M. Baumgart*[1], *M. Bott*[1]
[1]*Forschungszentrum Jülich, IBG-1, Jülich, Germany*
[2]*Technical University of Denmark, DTU BIOSUSTAIN, 2800 Kgs. Lyngby, Denmark*

Backgrounds

NADPH-dependent alcohol dehydrogenases (Adhs) play an important role in industrial biotechnology, especially for the production of chiral alcohols. A high-throughput *in-vivo* screening for these enzymes was established using suitable *Escherichia coli* reporter strains. The screening system is based on the genetically encoded NADPH biosensor pSenSox, which exploits the transcriptional regulator SoxR, its target promoter P_{soxS} and the reporter gene *eyfp*. It was shown that *E. coli* cells carrying pSenSox and expressing an NADPH-dependent *adh* of *Lactobacillus brevis* become fluorescent when the substrate methyl acetoacetate is reduced to (*R*)-methyl 3-hydroxybutyrate. Under suitable conditions, the specific fluorescence of the cells correlates with enzyme activity. Hence, the system enables high-throughput screening of large Adh libraries using fluorescence-activated cell sorting (FACS).

Objectives

As a prerequisite for potential improvements of the screening system, the NADPH biosensor was further characterized. Specifically, the influence of different growth media, hydrogen peroxide (H_2O_2), redox-cycling drugs (paraquat and menadione), the proposed SoxR reducing system (RseC and RsxABCDGE) and two transhydrogenases (SthA and PntAB) on the biosensor response was analyzed.

Methods

For the analysis of the biosensor response to altered conditions a BioLector® system was used. This system allows online recording of eYFP fluorescence and growth. HPLC analysis was used to confirm substrate biotransformation.

Conclusions

Except for H_2O_2, all other treatments influenced the sensor signal to different extent. These findings can be used for example to expand the dynamic range of the sensor, making it even more attractive and effective for the screening of NADPH-dependent Adh libraries.

FEMS7-1932
Biotechnology / Synthetic Biology / Systems Biology - Part III

CLONING XYLOSE REDUCTASE AND XYLITOL DEHYDROGENASE GENES FROM SPATHASPORA YEASTS ISOLATED FROM BRAZIL

A. Mouro[1], G.F. Gubert[1], A.A. dos Santos[1], B. Stambuk[1]
[1]Universidade Federal de Santa Catarina- Centro de Ciências Biologicas, Bioquímica, Florianopolis, Brazil

Backgrounds

We analyzed the fermentation of xylose and glucose by 32 yeast strains isolated in Brazil. Under comparative conditions, yeasts from the *Spathaspora* clade produced more ethanol from xylose than from glucose. The determination in the *Spathaspora* yeast strains of xylose reductase, xylitol dehydrogenase and xylulokinase activities showed that all strains presented NAD^+-dependent xylitol dehydrogenase activity, and *Sp. passalidarum* had the highest activity of this enzyme. Interestingly, *Sp. passalidarum* and *Sp. arborariae* showed xylose reductase activities with both NADH and NADPH cofactors.

Objectives

From the draft genome sequence of *Sp. arborariae* and *Sp. passalidarum* we cloned xylose reductases and a xylitol dehydrogenase, and expressed them in a *Saccharomyces cerevisiae* strain overexpressing the endogenous xylulokinase gene (*XKS1*).

Methods

While the cloned xylose reductase gene from *Sp. arborariae* accepted both cofactors, the enzyme cloned from *Sp. passalidarum* used only NADPH, indicating the existence of different enzymes in this yeast. The genes expressed in *S. cerevisiae* allowed xylose consumption by the cells, and improved consumption rates were observed if the *PHO13* gene was deleted from the strain.

Conclusions

Thus, *Spathaspora* yeasts may be considered a valuable platform for new genes encoding enzymes involved in xylose fermentation to engineer industrial *S. cerevisiae* yeasts.

FEMS7-3067
Biotechnology / Synthetic Biology / Systems Biology - Part III

WASTE PRODUCTS FROM POULTRY INDUSTRY – THE SOURCE OF BIOACTIVE COMPOUNDS

H. Stiborova[1], B. Branska[2], P. Patakova[2], M. Jiru[3], J. Poustka[3], K. Demnerova[1]
[1]University of Chemistry and Technology, Biochemistry and Microbiology, Prague, Czech Republic
[2]University of Chemistry and Technology, Biotechnology, Prague, Czech Republic
[3]University of Chemistry and Technology, Food Analysis and Nutrition, Prague, Czech Republic

Backgrounds

Millions of tons of raw feather and biomass after chicken mechanical deboning are produced each year by the poultry industry. Despite the fact that feather contains more than 90% of protein keratin is simply disposed of in incineration plants.

Objectives

The aim of this study was to i) transform the raw feather waste using three different approaches (whole cell microbial digestion, enzymatic and chemical cleavage) and to test the usage of the hydrolysates as peptone substitutes in culture medium.

Methods

Among all isolated keratin-degrading bacteria, *Pseudomonas* sp. P5 achieved the highest feather hydrolysis. Over 300 mg/L of free amino acids and 6.2 g/L of peptides were released from 90 g/L of wet raw feather by microbial hydrolysis. Hydrolysates obtained by semi-purified keratinase contained 1191 mg/L of amino acids and 3.3 g/L of peptides. The highest amount of peptides (17.2 g/L) was achieved by mild alkali condition and this hydrolysate also proved the best properties as cultivation medium (1).

Conclusions

All approaches tested could convert raw feather waste into products of commercial value with proven use in a cultivation medium. The level of peptides, their molecular size and amino acid composition was dependent on the method used.

Acknowledgement: This project was funded by the Technology Agency of the Czech Republic project BIORAF TE01020080.

1. Stiborova et al., J Chem Technol Biotechnol 2016, 9:1629-1637

FEMS7-3101
Biotechnology / Synthetic Biology / Systems Biology - Part III

THE LANDSCAPE OF MICROBIAL PHENOTYPIC TRAITS AND THEIR GENETIC BASIS

M. Brbic[1], M. Piskorec[1], V. Vidulin[1], A. Krisko[2], F. Supek[1,3]
[1]Institut Ruđer Bošković, Division of Electronics, Zagreb, Croatia
[2]Mediterranean Institute of Life Sciences, Macromolecular homeostasis in aging, Split, Croatia
[3]Centre for Genomic Regulation, EMBL/CRG Systems Biology Research Unit, Barcelona, Spain

Backgrounds

Bacteria and Archaea display a variety of phenotypic traits and can adapt to diverse ecological niches. While the amount of sequenced genomes is increasing rapidly, systematic phenotypic annotation is not keeping pace, meaning that the potential for comprehensive genome-phenome association studies is yet to be realized.

Objectives

We have developed ProTraits, a computational pipeline that annotates microbes with phenotypes by text-mining the scientific literature and the broader World Wide Web, while also being able to define novel concepts from unstructured text.

Methods

ProTraits draws extensively on comparative genomics, capturing evolutionary patterns in gene repertoires, codon usage biases, proteome composition and co-occurrence in metagenomes. Notably, we find that gene synteny is highly predictive of many phenotypes, and highlight examples of gene neighborhoods associated to spore-forming ability. The ~545,000 inferred phenotypes are provided with false discovery rate estimates validated by literature curation and are available at http://protraits.irb.hr/. A global analysis of trait interrelatedness reveals a lower-dimensional structure that underlies the microbial phenotype space, suggesting common genetic underpinnings.

Conclusions

The extended set of phenotypic annotations allows detection of 57,088 high confidence gene-phenotype links - a 6.6-fold gain over previous databases. We highlight examples of recovered associations involving known sporulation, flagellar and catalase genes, as well as pathways linked to phenotypes such as aerobicity and photosynthesis. Over 99% of commonly occurring gene families are involved in genetic interactions conditional on a phenotype, suggesting that epistasis has a major role in shaping microbial gene repertoires.

This work was published as Brbić et al. Nucleic Acids Res (2016) 44 (21): 10074-10090.
doi:10.1093/nar/gkw964

FEMS7-0833
Biotechnology / Synthetic Biology / Systems Biology - Part III

BIOTECHNOLOGICAL POTENTIAL OF CAVES STRAINS

O. Suslova[1], *O. Tashyrev*[1]
[1]*Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Biology of Extremophilic microorganisms, Kiev, Ukraine*

Backgrounds

Microbial biotechnologies became one of the most efficient ways to utilize wastewaters from mining or metal processing plants. The basic point of such technologies is bacterial strains that survive and interact with metals in high concentrations.

Objectives

During studies of microbial communities from Western Ukraine cave clays it was isolated two strains with high indexes of environmental plasticity – *Pseudomonas azothoformans* KC1 and *Rhodococcus erythropolis* P3. The aim was to analyze their survival rate during cultivation in the simultaneously presence of toxic metal (Cu^{2+} in the form of $CuCl_2$) and toxic organic compound (1-chloro-4-nitrobenzene - CNB).

Methods

Strains were cultivated in the NB medium (HiMedia Laboratories, India) with water solution of Cu^{2+} and alcohol solution of CNB in concentrations of 300 ppm (w/v). Optical density was controlled by spectrophotometer KFK-2MP (ZOMZ, Russia), the content of gas phase (CO_2, O_2, H_2) – by gas chromatography (LCM-8MD), the ability to transform CNB by mass-spectrum system Agilent 6890N/5973inert (capillary column HP-5MS (J&W Scientific, USA)), and the concentration of Cu^{2+} - by PAR analytical technique with titration by EDTA.

Conclusions

Both strains have shown the ability to survive in super high concentrations of organic and nonorganic xenobiotics – 300 ppm (compare 10 ppm is a well-known bactericidal concentration). Moreover, it was shown that even in such concentrations of toxic compound these strains could degrade CNB to nonaromatic compounds and extract up to 20% of Cu^{2+} from the media. Both bacterial strains were characterized by high adaptive potential and could be used in wastewaters treatment biotechnologies development.

FEMS7-0944
Biotechnology / Synthetic Biology / Systems Biology - Part III

ANALYSIS OF CHITINASE ACTIVITIES IN SMITTIUM FUNGI: PRODUCTION, ISOLATION, BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION

M. Takó[1], V. Bense[1], A. Kotogán[1], G. Nagy[2], Á. Csernetics[1,3], C. Vágvölgyi[1], T. Papp[1,2]
[1]University of Szeged, Department of Microbiology, Szeged, Hungary
[2]University of Szeged, HAS-USZ "Momentum" Fungal Pathogenicity Mechanisms Research Group, Szeged, Hungary
[3]Hungarian Academy of Sciences, -, Budapest, Hungary

Backgrounds

Smittium fungi (Kickxellomycotina) are living in the digestive tract of freshwater insect larvae. Although some strains were isolated, their physiology and enzyme production have not been investigated so far. And their relationship with the hosts is unknown. Chitinases are important in the biotechnology due to their ability to degrade chitin in the fungal cell wall and insect exoskeleton. This offers various applications as antimicrobial agents and chitinous waste degraders.

Objectives

The objective of our study was to analyze the chitinase production of five *Smittium* strains from Szeged Microbiological Collection. Then, biochemical and functional characterization of the promising chitinase activities. Until now, mostly bacterial chitinases were examined; therefore, our investigations would provide new chitinase producer fungal strains/enzymes.

Methods

Colloid chitin was used to induce the chitinase production. The enzyme was purified by ammonium sulfate precipitation, ion exchange and size exclusion chromatographies.

Conclusions

The *Smittium simulii* exhibited the highest chitinase yield. The molecular mass of its chitinase was about 60 kDa, and the optimum pH and temperature of the activity were pH 5.8 and 40 °C, respectively. The enzyme was stable between pH 5.4-6.6 and 10-50 °C, and in the presence of various cations, EDTA and SDS. The fungal cell wall-degrading activity of the chitinase was analyzed with the plant pathogenic fungus *Sclerotinia sclerotiorum*. The enzyme caused intensive vacuole formation in the mycelia after 24-h. This is the first chitinase from Kickxellomycotina, and results show that the enzyme is promising for biotechnological purposes. This research was supported by the NKFIH PD 112234 and GINOP-2.2.1-15-2016-00006.

FEMS7-1150
Biotechnology / Synthetic Biology / Systems Biology - Part III

THE SOLAR PANEL MICROBIOME: COMPOSITION, COLONISATION AND BIOTECHNOLOGICAL APPLICATIONS

K. Tanner[1], C. Vilanova[2], J. Peretó[1], M. Porcar[1]
[1]Institute for Integrative Systems Biology I2SysBio, Biotechnology, Paterna, Spain
[2]Darwin Bioprospecting Excellence SL, Bioprospecting, Paterna, Spain

Backgrounds

Bacterial communities are present in practically every natural and artificial environment. Up to date, many studies have addressed the microbial communities associated to natural habitats, but many artificial environments remain unexplored.

Objectives

This work aims at describing the bacterial communities associated to a high-temperature, highly irradiated and nutrient-poor artificial environment: solar panels. Furthermore, we aim to provide an insight on the biotechnological applications of the isolated bacterial strains and on the colonisation process of the glass panels.

Methods

By combining high-throughput 16S rRNA sequencing, metagenomic sequencing, metaproteomics, HPLC, *in vivo* assays and culture-based techniques, this research provides a holistic approach to the description of a previously unexplored environment.

Conclusions

Despite the harsh conditions, the sampled solar panels harboured a highly diverse microbial community rich in drought-, heat- and radiation-adapted bacterial genera. This bacterial community, similar to other highly-irradiated environments such as deserts or polar microbial mats, was also rich in carotenoid- and sphingolipid-producing bacteria, metabolites with potential biotechnological applications mainly as antioxidants or in UV-protecting creams. A further two-year colonisation experiment in which small solar panels will be sampled and analysed through 16S rRNA sequencing every seven weeks aims at elucidating the colonisation process of this artificial environment and the relationship of bacterial communities with photovoltaic efficiency.

FEMS7-2790
Biotechnology / Synthetic Biology / Systems Biology - Part III

USE OF WATER SOLUBLE EXTRACTS FROM ULVA SP. BY PROBIOTICS AND FISH BACTERIAL PATHOGENS

S.T. Tapia-paniagua[1], M. Fumanal[2], C. Fernández-Díaz[3], E. Clavijo[4], M.Á. Moriñigo[5], M.D.C. Balebona[5]
[1]Universidad De Malaga, Microbiologia, Malaga, Spain
[2]University Of Malaga, Microbiology, Malaga, Spain
[3]Instituto De Investigacion Y Formacion Agraria Y Pesquera, Acuicultura Y Recursos Marinos, Malaga, Spain
[4]University Of Malaga. Faculty Of Medicine, Microbiology, Malaga, Spain
[5]University Of Malaga, Microbiology, Malaga, Spain

BACKGROUNDS

The potential of seaweeds as dietary components is considered for a wide range of cultured fish species. In this context, *Ulva* is investigated as a good source of protein, minerals and vitamins. In addition, of probiotics are used to improve fish growth and modulate immune system and intestinal microbiota. To promote probiotics colonization and maintenance in the intestine, prebiotics are included in fish diets. Prebiotics are indigestible substrates used as energy sources for gastrointestinal microbiota, with a positive effect on the nutrition and health status of the host.

In the present work, ability of selected probiotic and fish pathogen strains to use water soluble extracts from *Ulva* as nutrient source has been evaluated.

MATERIALS AND METHODS

Water-soluble extracts from *Ulva* sp. prepared by sonication of dehydrated samples were used to supplement minimum medium (M9). Probiotics and pathogens growth was evaluated based on the optical densities measured with a microplate reader.

RESULTS AND CONCLUSIONS

Probiotics were able to grow in minimum medium using water soluble extracts as nutrient source. On the other hand, *P. damselae* subsp. *piscicida* and *V. harveyi* strains were also able to grow with *Ulva* extracts as nutrient source. However, incubation time to reach maximum growth was longer.

Although *Ulva* extract may support growth of both probiotics and pathogen bacteria, faster growth of probiotics may help for the establishment of probiotic populations in the intestinal environment. In addition, beneficial effects on growth performance, gut microbiota, immunity and disease resistance of *Ulva* for *Solea senegalensis* are being studied.

This work was funded by INIA, Ministerio de Economía y Competitividad and FEDER (RTA201400023 C0202).

FEMS7-1303
Biotechnology / Synthetic Biology / Systems Biology - Part III

USING BCG AS EXPERIMENTAL TREATMENT FOR ASTHMA IN MICE MODELS

R. Tarancon[1], S. Uranga[1], C. Martín[1], N. Aguiló[1]
[1]Universidad de Zaragoza, Microbiología- Medicina Preventiva y Salud Pública, Zaragoza, Spain

Backgrounds

Allergic diseases have reached epidemic proportions worldwide and its incidence continues increasing mainly due to the occidental lifestyle, generating huge economic burden. Therefore, it is essential to develop therapies targeting the cause of the disease and not just the symptoms, as is the case today (Stephen T. Holgate Nature Reviews.2015).

Objectives

The aim of this project is evaluating the impact of the potential treatment of asthma with BCG and other attenuated mycobacteria. This idea arose from the fact that mycobacteria triggers strong Th1 response that opposes the predominant Th2 response elicited in allergy.

This hypothesis has been tested in animal models (Carvalho Gouvela,J.Clin.Immunol.2013, Klaus Josef.Rockefeller University.1998) and in humans (Inseon Choi,Asthma Immunology.2002) using the BCG vaccine, with positive results.

Methods

We have developed a model for asthma induction in mice, using ovalbumin sensitization and challenge. We have also created a protocol to quantify eosinophils in the bronchoalveolar fluid (BAL) by flux cytometry. We have analyzed cytokines in BAL and in lung explants with the ELISA technique.

Conclusions

The analysis of BAL from mice treated with the allergen ovalbumin reflects a clear eosinophilic and interleukin-5 infiltration with respect to negative controls. The intranasal treatment with BCG after ovalbumin sensitization decreases eosinophilic and interleukin-5 concentration in BAL, decreases the interleukin-4 and increases the interferon-γ in lung explants compared to untreated animals.

We also demonstrate that the vaccine has a long-term protection against asthma.

These results are encouraging and suggest that BCG and other attenuated mycobacteria could be potential candidates for treatment of allergic asthma.

FEMS7-1692
Biotechnology / Synthetic Biology / Systems Biology - Part III

MARINE BACTERIA AS A PROLIFIC SOURCE FOR NOVEL BIOCATALYSTS

A. Bollinger[1], S. Thies[1], C. Coscolín[2], N. Katzke[1], S. Söltl[3], J. Navarro-Fernández[2], P. Golyshin[4], M. Ferrer[2], K.E. Jaeger[1]
[1]Heinrich-Heine-University Düsseldorf, Institute for Molecular Enzyme Technology, Juelich, Germany
[2]Consejo Superior de Investigaciones Científicas, Institute of Catalysis, Madrid, Spain
[3]Fachhochschule Aachen, Campus Jülich, Juelich, Germany
[4]Bangor University, School of Biological Sciences, Bangor, United Kingdom

Backgrounds

The demand for novel biocatalysts fitting industrial requirements is rapidly increasing. As it is accepted that the natural habitat of a given host organism shapes the properties of its enzymes, and that marine habitats are subject to dramatic variability, marine metagenomics can be expected to deliver novel biocatalysts with interesting properties.

Objectives

As part of the EU Horizon 2020 project “Industrial Applications of Marine Enzymes” (INMARE), we have mined the virtually untapped richness of marine microorganisms with different strategies to efficiently recover industrially relevant biocatalysts with a particular focus on transaminases and hydrolases that are among the two most important groups of biocatalysts for chemical synthesis.

Methods

Promising candidate biocatalysts were identified by a combination of *in silico* genome mining and activity-based functional screening. Screened libraries were constructed from metagenomic DNA[1] and genomic DNA obtained from hydrocarbonoclastic bacteria isolated from marine environments. Established and newly developed activity-based high-throughput screening strategies were applied allowing for the rapid determination of enzyme function and substrate specificity. Among other enzymes, 18 novel transaminases and a highly substrate promiscuous esterase-lipase, stable at 45°C and up to 40% of organic solvents, from *Alcanivorax borkumensis*, were identified. From a practical standpoint, data are provided that suggest that some of the versatile and robust marine-derived biocatalysts may compete with the best industrial prototypes.

Conclusions

Marine metagenomic libraries as well as genomes of hydrocarbonoclastic bacteria represent a prolific source for novel biocatalysts with industrially relevant properties.

[1] Popovic *et al*., (2015). Adv Exp Med Biol 883, 1–20.

FEMS7-1746
Biotechnology / Synthetic Biology / Systems Biology - Part III

ACTINOALLOMURUS, A GENUS WITH THE POTENTIAL FOR NOVEL ANTIMICROBIALS AND BIOACTIVE MOLECULES

A. Tocchetti[1], M. Iorio[1], J.C.S. Cruz[2], C. Brunati[2], S.I. Maffioli[1,2], M. Sosio[1,2], S. Donadio[1,2]
[1]Naicons Srl, Milano, Italy
[2]Ktedogen Srl, Milano, Italy

Backgrounds

The emergence of a dramatic landscape of infectious diseases in these past few years clearly calls for a constant and significant investment on drug discovery, as the spread of antibiotic resistance keep occurring by giving origin to more multi-drug resistant strains.

One possibility to enhance the antibacterial arsenal is to look into what Nature has been using to solve the inevitable phenomenon of antibiotic resistance. The idea is to screen the myriad of microbial species collected from different natural environments, giving priority to those less exploited but phylogenetically related to known producers.

Objectives

The metabolic potential of the promising genus *Actinoallomurus* was accessed by a screening program based on the dereplication process of a small selected portion of Naicons' library (ca. 200 *Actinoallomurus* microbial strains).

Methods

Small-scale fermentations were followed by cell-based bioactivity assays of the broths extracts. Positive samples were then subjected to a combination of chemical analyses and queries within a proprietary natural product database to determine the novelty of active compounds. The data obtained show that *Actinoallomurus* can produce a broad range of chemical classes, from lantipeptides to new hyperhalogenated angucyclines or new aromatic polyethers. Few examples of these will be presented.

Furthermore, genomic analysis of some interesting *Actinoallomurus* strains revealed the presence of a diverse set of clusters for secondary metabolites, corroborating the biosynthetic potentiality of this genus.

Conclusions

In conclusion, both cultivation screening and genomic analysis of *Actinoallomurus* supported this approach for the discovery of new interesting bioactive metabolites.

FEMS7-2732
Biotechnology / Synthetic Biology / Systems Biology - Part III

INTEGRATIVE ANALYSIS OF THE FITNESS EFFECTS OF PLASMIDS IN PSEUDOMONAS AERUGINOSA PAO1

M. Toll Riera[1], *A. San Millan*[2], *Q. Qi*[3], *C. MacLean*[4]
[1]*University of Zürich, Deparment of Evolutionary Biology and Environmental Studies, Zürich, Switzerland*
[2]*Hospital Universitario Ramon y Cajal IRYCIS, Department of Microbiology, Madrid, Spain*
[3]*University of Cologne, Institute for Theoretical Physics, Cologne, Germany*
[4]*University of Oxford, Department of Zoology, Oxford, United Kingdom*

Backgrounds

Plasmids mediate the horizontal transmission of genetic information between bacteria, facilitating their adaptation to multiple environmental conditions. Plasmids confer new adaptive tools to bacteria, but they also entail a metabolic burden that reduces the competitive ability of the plasmid-carrying clone in the absence of selection for plasmid-encoded traits. This reduction in fitness is the main constraint to the vertical and horizontal replication of these genetic elements. However, the molecular bases underlying the fitness costs produced by plasmids remain poorly understood.

Objectives

Our objective is to gain a better understanding of the bacterial response to the fitness cost caused by plasmids in the bacterium *P. aerugionsa PAO1*.

Methods

We introduced five different natural plasmids (three clinical, one isolated from soil and one isolated from waste water) into *P. aeruginosa* PAO1 and we combined an array of techniques (fitness assays, transcriptomics and metabolomics) to better understand how *P. aeruginosa* responds to the fitness costs

Conclusions

Preliminary results suggest that PAO1 differentially expresses a different set of genes when exposed to each plasmid. However, there is a common subset of genes, with an over-representation of genes involved in bacterial metabolism, which is differentially expressed in response to most plasmids. Finally, some hints suggest that the fitness cost caused by the plasmids might be explained by the difference in the codon usage with respect to *P. aeruginosa*, being the cost higher when the difference in codon usage is bigger.

FEMS7-1187
Biotechnology / Synthetic Biology / Systems Biology - Part III

HIGHER ANTIOXIDANT DEFENCES RESULT IN A BETTER INDUSTRIAL PERFORMANCE IN NON-SACCHAROMYCES WINE YEASTS

M. Torrellas[1], *E. Matallana*[1], *A. Aranda*[2]
[1]*Universitat de Valencia, Bioquímica y Biología Molecular, Valencia- Burjassot, Spain*
[2]*Instituto de Agroquímica y Tecnología de Alimentos CSIC, Biotecnología de Alimentos, Paterna, Spain*

Backgrounds

In recent years there has been a growing interest in the usage of non-*Saccharomyces* yeasts as co-inoculums in the wine industry. There have been many reports in which different species have been used in mixed fermentations alongside *Saccharomyces cerevisiae*. Their ability to produce secondary metabolites, reduce alcohol content or their specific enzymatic activities affect the wine profile, resulting in wines with enhanced and more complex flavours and aromas, which makes them important biotechnological tools. Most of these non-*Saccharomyces* yeasts occur naturally in grapes and wine environments, and although there are already commercially available strains, most inoculums have not been produced at an industrial scale.

Objectives

We aim to characterize the behaviour and redox state of a set of non-*Saccharomyces* yeasts during yeast biomass propagation and dehydration, the two main industrial processes in active dry yeast production. We also intent on understanding the effects of the oxidative stress associated with these processes in their industrial performance.

Methods

In this study we used a set of biochemical and physiological parameters that allowed us to determine the redox state of the studied yeasts, amongst them we analysed trehalose and gluthatione levels, gluthatione reductase and catalase activity, and lipid peroxidation. In order to analyse the industrial performance of our set of yeasts we measured the fermentative capacity and their viability after dehydration.

Conclusions

Our results show high variability between the studied yeasts, those with increased antioxidant defences present a better tolerance and a reduction in fermentative capacity loss after dehydration, due to reduced oxidative damage.

FEMS7-0668
Biotechnology / Synthetic Biology / Systems Biology - Part III

COMPARISON OF EFFECTIVENESS OF ROSEOFUNGIN-AS, 2% OINTMENT, AND CLOTRIMAZOLE, 1% CREAM, IN THE TREATMENT OF FUNGAL SKIN DISEASES: A RANDOMIZED, MULTICENTRE, DOUBLE-BLIND CLINICAL TRIAL

L. Trenozhnikova[1], A. Balgimbaeva[1], G. Ultanbekova[1], V. Berezin[1], A. Sadanov[1]
[1]Institute of Microbiology and Virology, Department of Microbiology, Almaty, Kazakhstan

Backgrounds

Despite the variety of forms, the therapeutic arsenal of antifungal drugs for local application is limited, and there is a clear need for new, more effective and less toxic antifungal agents.

Objectives

The antifungal drugs Roseofungin-AS, 2% ointment, and Clotrimazole, 1% cream.

Methods

The safety and effectiveness of antifungal drugs were compared in a multicenter, randomized, double-blind, parallel-group trial involving 466 patients. The drugs were used topically applying to the skin surface twice a day for 28 days.

Conclusions

A new representative of the polyene group of antibiotics - carbonyl-conjugated pentaen roseofungin – is an active ingredient of Roseofungin-AS, 2% ointment. The single- and multiple-dose application of Roseofungin-AS, 2% ointment did not produce any toxic effects. High effectiveness of Roseofungin-AS was established in dermatomycoses of different locations - athlete's foot and smooth skin mycoses caused by *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton verrucosum*, *Microsporum gypseum*. Clinical recovery of patients was observed 2 weeks after using Roseofungin-AS, 2% ointment, in the treatment of smooth skin mycoses and 3 weeks after using it in the treatment of athlete's foot. Clotrimazole supported a regression of clinical signs following more prolonged application: during 3 weeks in the treatment of skin smooth mycoses and 4 weeks in the treatment of athlete's foot. In the open, prospective, randomized Phase II/III trial high clinical and mycological effectiveness of the antifungal agent Roseofungin-AS was established in patients with fungal skin infections as compared with the drug Clotrimazole for external use - 97.4% and 93.6%, respectively.

FEMS7-0357
Biotechnology / Synthetic Biology / Systems Biology - Part III

OPTIMIZATION OF A FAST AFFINITY ADSORPTION PURIFICATION METHOD OF FUNCTIONAL LPMOS

S. Valenzuela[1], *G. Ferreres*[1], *G. Margalef*[1], *M. Rodríguez-Astigarraga*[1], *F.I.J. Pastor*[1]
[1]*University of Barcelona, Department of Genetics- Microbiology and Statistics, Barcelona, Spain*

Backgrounds

The recently discovered LPMOs are crucial enzymes for the deconstruction of recalcitrant macromolecules, including lignocellulosic and chitin polymers, thus represent a promising component of industrial cocktail enzymes for degrading biomass. The LPMOs are metalloenzymes that oxidize glycosidic bonds and the catalysis requires the binding of an active oxygen molecule to the copper atom of the enzyme for the oxidative depolymerization of the substrate. Depending on the type of LPMO, the products released are gluco-oligosaccharides that are oxidized either at the reducing (C1) or in both, the reducing (C1) and the non-reducing (C4) end (3).

Objectives

To develop a simple and low-cost method for recombinant LPMOs purification.

Methods

We have cloned two orfs encoding for putatives LPMOs from *Streptomyces ambofaciens*, which is a soil bacterium industrially exploited for the production of antibacterial chemicals. We established a purification method for these enzymes based on the ability of the recombinant LPMOs to bind polysaccharides. In addition, one of the produced LPMOs, named SamAA10C was tested against a variety of cellulosic substrates, including for the first time for an LPMO, bleached pulps used in the paper industry.

Conclusions

We developed a simple and fast method to purify LPMOs in a single step without using any chromatographic column or periplasmic preparation, considerably reducing time used in others studies for the purification step. The purification method introduced for the LPMOs could be a cheap solution, to use these enzymes in industrial applications, and the activity of SamAA10C show the potential of LPMOs in biomass upgrading.

FEMS7-2811
Biotechnology / Synthetic Biology / Systems Biology - Part III

BIOINFORMATIC SCREENING OF NOVEL ENZYMES FOR BIOMASS REVALORITZATION

P. Soteras[1], F.I.J. Pastor[1], S. Valenzuela[1]
[1]Universitat de Barcelona, Microbiology, BARCELONA, Spain

Backgrounds

The strain BP-23 isolated from soil presents a high depolymerizing activity of vegetal biomass due to its capability to degrade polysaccharides. One of the most relevant activity of the strain is xylanase. This activity allows the strain to grow in minimum medium with xylan, the second most abundant polysaccharide in the earth, as a unique source of carbon.

Objectives

The main objectives are to characterize all the xylanases involved in the xylanolytic system of the strain and to identify all the proteins involved in the degradation of this substrate.

Methods

As a first stage, the sequencing of the genome of the strain has been performed. Then a multiple alignment of sequences encoding known enzymes with the genome of the studied strain allowed the identification of several DNA segments showing homology to known genes. DNA segments with the best score were chosen and analysed their upstream and downstream sequences to identify putative *orfs*. As a fourth stage, a BLASTx focusing on microorganisms taxonomically related to BP23 was performed, in order to select those *orfs* with homology to genes encoding relevant enzymes. Specific primers were designed to amplify these *orfs* by PCR, which were cloned in expression vectors and transformed in *E. coli.* The recombinant strains obtained were cultured to produce the new enzymes, which were purified and characterized.

Conclusions

Multiple new *orfs* have been identified and most of them codify for active proteins. Among them, several new xylanases, expansins, celulases, esterases and pectatolyases from the strain have been characterized by the research group.

FEMS7-2011
Biotechnology / Synthetic Biology / Systems Biology - Part III

HETEROLOGOUS EXPRESSION AND MODIFICATION OF ANTIMICROBIAL PEPTIDES FROM EUKARYOTES WITH LANTIBIOTIC BIOSYNTHETIC ENZYMES IN LACTOCOCCUS LACTIS

S.B. van der Meulen[1], *A. de Jong*[1], *O.P. Kuipers*[1]
[1]*University of Groningen, Molecular Genetics, Groningen, The Netherlands*

Backgrounds

Due to an ever increasing resistance of bacteria to available antibiotics, new types of antimicrobial molecules need to be developed and discovered. We focus on the mining of antimicrobial peptides from eukaryotic organisms, which are subsequently re-designed, heterogeneously produced and post-translationally modified in our expression host *Lactococcus lactis*.

Objectives

We aim to create highly stable lantibiotic peptides that are currently not produced in nature.

Methods

Mining for suitable candidate peptides was performed from more than 20.000 peptide sequences for the presence of a disulfide bridge and applicability for post-translational modification by the nisin biosynthesis machinery. Six peptides of 10-17 AA in length were selected and re-designed to enable lanthionine ring formation. The peptides were expressed in combination with the proteins that dehydrate serine and threonine residues (NisB), enable cyclization (NisC) and peptide transport (NisT). Activity tests have shown that two peptides are in particular active against *Lactococcus lactis*, *Micrococcus flavus* and *Streptococcus pneumoniae*. The production was scaled up to 1L batch production, followed by His-tag purification on NGC, HPLC and analysis on a mass spectrometer. This work is ongoing to proof dehydration and lanthionine ring formation.

Conclusions

We have successfully produced bioactive peptides that are derived from different eukaryotic organisms. Although the production of some of the peptides remains challenging, two promising peptides show a potent activity against various Gram-positive bacteria. We are currently working on purification of the peptides to enable quantitative tests, toxicity assays and to gain insight into the mode of action of the peptide.

FEMS7-0631
Biotechnology / Synthetic Biology / Systems Biology - Part III

CRSIPRI FOR OPTIMIZED ECTOINE PRODUCTION IN HALOMONAS ELONGATA

J. Vandrich[1], G. Alfaro Espinoza[1], H.J. Kunte[1]
[1]Federal Institute for Materials Research and Testing BAM, Materials and the Environment, Berlin, Germany

Backgrounds

Halophilic bacteria synthesize and accumulate organic osmolytes to cope with osmotic stress in saline environments. Ectoine is an important osmolyte synthesized by many halophilic bacteria. It is produced by industry because it possesses protecting properties and stabilizes proteins, membranes and whole cells against stresses such as UV radiation and cytotoxins.

Objectives

Today, industrial production processes rely on the halophilic bacterium *Halomonas elongata* and output ectoine on an annual scale of tons, which is then processed into cosmetics and pharmaceuticals. Still, the factors for optimum ectoine production remain to be fully explored. Therefore, we are establishing the CRISPR-Cas9 system in *H. elongata* DSM 2581^T to investigate basic metabolic pathways and subsequently optimize ectoine production.

Methods

The CRISPR-Cas9 system is a versatile molecular tool that allows simultaneous introduction of multiple genetic modifications. We are using the repurposed CRISPRi (CRISPR interference) variant for sequence-specific gene repression in *H. elongata*. The catalytically inactive dCas9 was integrated into the genome under the constitutive expression of the *teaABC* operon. Accurately designed guideRNA constructs were added to trigger site-directed repression of target genes.

Conclusions

The first target gene was one of the ectoine synthesis genes, *ectA*. The repression of *ectA* resulted in an easy-to-observe decrease of ectoine production. Additionally, changes in gene expressions levels were determined precisely via qRT-PCR measurements.This successfully established CRISPRi system will not only allow us to further investigate ectoine metabolism but it will also be a valuable tool for genomic research in *H. elongata* e.g. on the preferred glycolytic strategies under varying salt concentrations.

FEMS7-0986
Biotechnology / Synthetic Biology / Systems Biology - Part III

AN EXPEDITION INTO THE NEGLECTED HUMAN "MICROBIAL" ORGAN

L. Vasudevan[1], J. V[2], S. M.[2], C. T. S.[1]
[1]Indian Institute of Technology Madras, Department of Biotechnology, Chennai, India
[2]Global Hospital and Health City, Gastroenterology, Chennai, India

Backgrounds

'Gut microbiota' refers to the ecosystem of microorganisms that have adapted to live on intestinal mucosal surface or within gut lumen (WGO Handbook of gut microbes, 2014). Gut flora has received renewed research interest for potential associations with human health. Distortions in any of the microbiota functions result in a wide range of diseases. Hence it has earned the status of human "microbial" organ.

Microbial composition varies along the length of gastrointestinal tract. Use of tissue samples provides better expression of autochthonous bacterial population well-adapted to gut and they constitute better probiotic strains than allochthonous (foreign) strains.

Objectives

To exploit gut microbes for health benefits by isolating and characterizing the bacterial species inhabiting our gut.

Methods

Biopsy samples were collected from three different regions of colon (terminal ileum, caecum and recto-sigmoid) from healthy volunteers. They were homogenized, enriched and plated. The isolates were subjected to molecular fingerprinting (BOX PCR-RAPD) which helped to categorize them. Representative isolates were identified and subjected to probiotic characterization (acid and bile tolerance, antimicrobial activity, aggregation ability and vitamin biosynthesis).

Conclusions

Key observations include
i. Enrichment helps in increasing the number of surviving bacteria.
ii. Anaerobic enrichment gives higher colony number.
iii. Striking difference observed in colony numbers obtained under aerobic and anaerobic enrichment.
iv. Colony diversity, however, does not vary significantly
   a. Among the three regions – ileum, caecum, and recto-sigmoid.
   b. Between aerobic and anaerobic enrichments.
v. They were able to withstand acid and bile conditions similar to that found in human system.
vi. The strains differed in their antimicrobial activity and ability to produce vitamin.

The isolated strains represent autochthonous gut population with higher probiotic functionality (in terms of ecological competence, ability to thrive and produce beneficial metabolic products) as compared to those reported from faeces and/or dairy products, and hence can be exploited for overall health management.

FEMS7-2423
Biotechnology / Synthetic Biology / Systems Biology - Part III

WASTE OILS AS AN ALTERNATIVE INDUCTOR TO PRODUCE LIPASES BY SUBMERGED CULTURE OF FILAMENTOUS FUNGI

M.V. Tacin[1], A.V. de Paula[2], V.D.C.S. Ebinuma[2]
[1]School of Pharmaceutical Sciences of São Paulo State University-UNESP, Food and Nutrition, Araraquara, Brazil
[2]School of Pharmaceutical Sciences of São Paulo State University-UNESP, Bioprocess and Biotechnology, Araraquara, Brazil

Backgrounds

The production of enzymes by fungi has been highlighted in biotechnology due to the process simplicity. Fermentation parameters such as nitrogen and carbon sources are factors that influence both the characteristic of enzyme produced and the biomolecule final cost. Regarding to lipases production, oil is used as an inductor during the biosynthesis. Industries of food and textile generate oils rich in linoleic acid such as grape and cottonseed oil as waste that can be used to this purpose.

Objectives

The main goal on this work was to evaluate grape and cottonseed oil as an alternative inductor to produce lipase by *Aspergillus tubingensis* and improve its production employing statistic tools.

Methods

Grape oil promoted the highest production of lipases according the enzymatic activity and it was used in the following experiments. So, the variables bacteriological peptone, glucose and grape oil concentration were studied through a 2^3 full factorial design followed by two 2^2 central composite factorial design. The regression analysis pointed out that bacteriological peptone and glucose were the variables that influenced more in the lipase production. Under the best conditions of the second 2^2 central composite factorial design, bacteriological peptone was the variable that presented more significance at a confidence interval of 95%.

Conclusions

In the best experimental condition it was achieved an increase in the enzymatic activity around 30%. In this way, grape oil can be used to promote the production of lipases by filamentous fungi and the factorial experimental design demonstrated an important role in the improvement of enzymes production.

FEMS7-2434
Biotechnology / Synthetic Biology / Systems Biology - Part III

PRODUCTION OF LIPASES BY ASPERGILLUS TUBINGENSIS EMPLOYING SUBMERGED CULTURE

M.V. Tacin[1], A.V. de Paula[1], V.D.C.S. Ebinuma[1]
[1]School of Pharmaceutical Sciences of São Paulo State University-UNESP, Bioprocess and Biotechnology, Araraquara, Brazil

Backgrounds

Filamentous fungi are recognized as good lipases producer and to improve the production conditions such as nutrients sources, fermentation time, among others are important to make microbial lipases competitive in the market.

Objectives

The objective of this work was to evaluate the production of lipases by submerged culture of *Aspergillus tubingensis*.

Methods

The fermentation was proceed in orbital shaker at 150 rpm at 30°C in Erlenmeyers flaks (500 mL) containing 10 mycelial agar disc and 100 mL of culture medium composed of: 20.0 g/L bacteriological peptone, 8.0 mL/L grape oil, 0.6 g/mL $MgSO_4.7H_2O$, 1.0 g/L KH_2PO_4, 1.0 g/L NH_4NO_3. The experiments were conducted in duplicate, being samples taken daily during 5 days of culture. At the end of the culture, the fermented was filtered and the biomass was quantified by dry weight while the liquid fermented was used to determine the lipase enzymatic activity using p-nitrophenyl palmitate as substrate, protein by Lowry and pH.

Conclusions

The initial pH and biomass was 6.34 and 0.56 g/L, respectively. After 3 days of bioprocess, the pH of the medium remained between 3.46 and 3.77 due to the action of metabolites produced by the microorganism. The highest production of lipase was obtained after 4 days of culture being biomass of 9.70 g/L and enzymatic activity was of 20.74 U/mL (specific activity of 6.80 U/mg protein), with an average increase of 25% in activity per daily production. With these results, it can be concluded that 4 days of submerged culture generate lipase with the highest activity.

FEMS7-2729
Biotechnology / Synthetic Biology / Systems Biology - Part III

RANDOM STREAMLINING OF THE E. COLI GENOME

V. Vernyik[1], *Z. Gyorfy*[1], *G. Posfai*[1]
[1]*Biological Research Center, Institute of Biochemistry, Szeged, Hungary*

Backgrounds

Creation of bacteria with randomly reduced genomes would allow better understanding of causes and consequences of reductive genome evolution. Moreover, cells with streamlined genomes would provide dependable chassis for synthetic biology. Current approaches mostly use targeted genome reduction, relying on *a priori* knowledge of gene function. An efficient tool for random genome streamlining, however, coupled to selection of the fittest population, is still missing.

Objectives

We aimed to develop a novel scheme to generate random deletions in a large population of *E. coli* in a cyclic and efficient manner.

Methods

Steps of the iterated deletion cycles are: (1) Random genomic integration of a transposon cassette, which carries an antibiotic resistance gene, a negative selection marker and unique I-SceI recognition sites. (2) Generation of double-strand DNA breaks by induction of I-SceI expression. In the survivor cells the DNA break is repaired by the alternative end-joining mechanism, which is associated with the formation of random deletions. (3) Elimination of background by negative selection.

Conclusions

The proposed random deletion generating method is feasible: deletions of various size (up to tens of kbp) can be efficiently created at random genomic sites. The process can be applied to a large population of bacteria, allowing, in principle, selection of the fittest variants.

FEMS7-2384
Biotechnology / Synthetic Biology / Systems Biology - Part III

SURVIVAL STRATEGIES FOR PROKARYOTES LACKING ESSENTIAL DNA REPAIR FACTORS

B.G. Vertessy[1], V. Grolmusz[2], C. Kerepesi[2], J. Szabó[3]
[1]Budapest University of Technology and Economics, Dept of Applied Biotechnology, Budapest, Hungary
[2]Eötvös University, Dept Computer Science, Budapest, Hungary
[3]Research Center for Natural Sciences, Inst Enzymology, Budapest, Hungary

Backgrounds

Enzymes involved in maintaining genome integrity are expected to be ubiquitous and essential for viability. Such an enzyme is dUTPase, that is required for maintaining the correct low level of dUTP, hence preventing dUMP (uracil) incorporation into DNA. Lack of dUTPase leads to drastic increase in uracil-DNA levels and prompt cell death via overloading the uracil-DNA glycosylase - base-excision repair pathway and inducing DNA fragmentation. We, however, found that Staphylococcus aureus does not encode its own dUTPase and seems to rely only on phage infection to acquire phage-encoded dUTPases.

Objectives

The intriguing situation in Staphylococcus led us to examine all genomes published for bacteria and Archea.

Methods

We have performed a bioinformatic screen on all available prokaryte genomes to decide if these encode dUTPase and uracil-DNA glycosylase genes, or genes for inhibitory proteins of dUTPases or uracil-DNA glycosylases. We also determined the uracil-DNA level in selected Staphylococcal samples.

Conclusions

We have shown that the genes for the common dUTPase enzyme families are far from being ubiquitous in prokaryotes. This unexpected genotype is observed in evolutionary well-separated branches suggesting that loss of the *dut* gene(s) might have occurred on multiple independent occasions during evolution. We also observe that elements involved in uracil-DNA metabolism are interestingly found within mobile genetic elements. The biomedical significance of these findings are especially relevant for microbes of current high therapeutic challenge.

FEMS7-1168
Biotechnology / Synthetic Biology / Systems Biology - Part III

THE USE OF GLYCEROL AS A CARBON SOURCE FOR THE PRODUCTION OF COLD-ACTIVE B-GALACTOSIDASES BY RECOMBINANT PICHIA PASTORIS YEAST STRAINS

M. Wanarska[1], J. Barański[1], E. Krajewska[1], M. Wicka-Grochocka[1], H. Cieśliński[1]
[1]Gdańsk University of Technology, Department of Molecular Biotechnology and Microbiology, Gdańsk, Poland

Backgrounds

A methylotrophic yeast *Pichia pastoris* is frequently used as a host for the production and secretion of a wide variety of recombinant proteins. It can utilize glucose, glycerol and methanol as a carbon and energy source. Efficient utilization of crude glycerol, a by-product from biodiesel production, could bring significant economic and environmental benefits. β-Galactosidase catalyzes decomposition of β-galactosides such as lactose by hydrolyzing O-glycosidic bonds. Due to this activity it is used in the food industry for the production of dairy products with reduced lactose content.

Objectives

The purpose of this study was to evaluate the possibility of using pure glycerol as well as crude glycerol derived from biodiesel production as a carbon source in growth medium for recombinant *P. pastoris* yeast strains producing cold-active β-galactosidases.

Methods

Recombinant *P. pastoris* strains capable of producing and secreting β-galactosidases derived from psychrotolerant bacteria *Arthrobacter* sp. S3*, *Arthrobacter* sp. 32cB and *Paracoccus* sp. 32d were cultivated in media containing three different carbon sources: glucose, pure glycerol and crude glycerol. The activity of enzymes in post culture media was measured using *o*-nitrophenyl β-D-galactopyranoside as a substrate.

Conclusions

The cell growth and protein expression profiles were similar for glucose and analytical grade glycerol. The yield of biomass was slightly higher for crude glycerol. The activity of β-galactosidases in post culture media was lower for crude glycerol than for glucose and pure glycerol. However, these results demonstrate the potential of using crude glycerol as the sole carbon source in the production of recombinant proteins in *P. pastoris* expression system.

FEMS7-2989
Biotechnology / Synthetic Biology / Systems Biology - Part III

IMPACT OF RRN OPERON DELETIONS ON GROWTH PARAMETERS OF THE AMINO ACID PRODUCER CORYNEBACTERIUM GLUTAMICUM

S. Weinmann[1], T. Hahn[1], M. Ruwe[2], J. Kalinowski[2], B. Eikmanns[1]
[1]Institute of Microbiology and Biotechnology, AG Eikmanns, Ulm, Germany
[2]Center for Biotechnology CeBiTec- Bielefeld University, CeBiTec, Bielefeld, Germany

Backgrounds

Bacterial 5S, 16S, and 23S ribosomal RNAs are encoded by the so-called *rrn* operons. There is a distinct number of *rrn* copies in each bacterium; *Escherichia coli* and *Corynebacterium glutamicum* possess seven and six *rrn* copies, respectively, whereas slow-growing *Mycobacterium* species contain only one or two *rrn* operons. Previous studies with *E. coli* indicated a correlation between the number of ribosomes and growth rate, the number of ribosomes being probably dependent on the availability of ribosomal proteins and rRNAs.

Objectives

Our project aims to analyze expression of individual *rrn* operons in *C. glutamicum* and to investigate the impact of the total chromosomal *rrn* copy number on various growth parameters of this organism.

Methods

Using a reporter gene system, the activities of all six *rrn* promoters were determined. Single and multiple *rrn* deletion mutants of *C. glutamicum* CR099 (up to five *rrn* operons deleted) were constructed and tested for growth rate, biomass, protein content, glucose consumption rate, cell size, and glutamate production.

Conclusions

The results indicate that the six *rrn* operons in *C. glutamicum* are differentially expressed and that there is negligible impact of *rrn* deletions on biomass, protein content, cell size, or glutamate production. However, when five of the six *rrn* operons were deleted, the growth and glucose consumption rates were impaired up to 40 %. These results show that *C. glutamicum* can compensate the loss of up to four *rrn* operons by so far unidentified mechanisms.

Acknowledgement

This work was supported by the BMBF grant 031A302E.

FEMS7-2930
Biotechnology / Synthetic Biology / Systems Biology - Part III

ETHYL ACETATE PRODUCTION BY THE ELUSIVE ALCOHOL ACETYL TRANSFERASE FROM YEAST

A. Kruis[1], M. Levisson[2], A. Mars[2], M. van der Ploeg[2], F. Garces Daza[1], V. Ellena[1], S. Kengen[1], J. van der Oost[1], R. Weusthuis[3]
[1]Wageningen University, Laboratory of Microbiology, Wageningen, The Netherlands
[2]Wageningen Research, Biobased Products, Wageningen, The Netherlands
[3]Wageningen University, Bioprocess Engineering, Wageningen, The Netherlands

Backgrounds

Ethyl acetate is an industrially relevant ester that is currently produced exclusively through unsustainable processes. Many yeasts are able to produce ethyl acetate, but the main responsible enzyme has remained elusive, hampering the engineering of novel production strains.

Objectives

Here we describe the discovery of a new enzyme (Eat1) from the yeast *Wickerhamomyces anomalus.*

Methods

When expressed in *Saccharomyces cerevisiae* and *Escherichia coli* it resulted in high ethyl acetate production. Purified Eat1 showed alcohol acetyltransferase activity with ethanol and acetyl-CoA. Homologs of *eat1* are responsible for most ethyl acetate synthesis in known ethyl acetate-producing yeasts, including *S. cerevisiae*, and are only distantly related to known alcohol acetyltransferases.

Conclusions

Eat1 is therefore proposed to compose a novel alcohol acetyltransferase family within the α/β hydrolase superfamily. The discovery of this novel enzyme family is a crucial step towards the development of biobased ethyl acetate production and will also help in selecting improved *S. cerevisiae* brewing strains.

FEMS7-2971
Biotechnology / Synthetic Biology / Systems Biology - Part III

MONASCUS RUBER AS CELL FACTORY FOR LACTIC ACID PRODUCTION AT LOW PH

R. Weusthuis[1]
[1]*Wageningen University, Bioprocess Engineering, Wageningen, The Netherlands*

Backgrounds

Lactic acid is a microbial product traditionally used to conserve food and feed products. Lactic acid is also used in technical applications, e.g. as building block of poly lactic acid (PLA). Lactic acid is inhibitory for microorganisms, especially at low pH values. The general strategy followed is to use well-known yeast cell factories and to convert them into lactic acid producers. Lactic acid tolerance is a complex trait and therefore difficult to engineer.

Objectives

We followed an alternative approach in which we searched for lactic acid tolerant microbial species in natural habitats and converted the most tolerant strain into a lactic acid producer.

Methods

A *Monascus ruber* strain was isolated that was able to grow on mineral medium at high sugar concentrations and 175 g/l lactic acid at pH 2.8. Its genome and transcriptomes were sequenced and annotated. Genes encoding lactate dehydrogenase were introduced to accomplish lactic acid production and two genes encoding pyruvate decarboxylase were knocked out to subdue ethanol formation. The strain preferred lactic acid to glucose as carbon source, which hampered glucose consumption and therefore also lactic acid production. Lactic acid consumption was stopped by knocking out 4 cytochrome-dependent genes, and evolutionary engineering was used to increase the glucose consumption rate.

Conclusions

Application of this strain in a fed-batch fermentation resulted in a maximum lactic acid titer of 190 g/l at pH 3.8 and 129 g/l at pH 2.8, respectively 1.7 and 2.2 times higher than reported in literature before. Yield and productivity were on par with the best strains described in literature for lactic acid production at low pH so far.

FEMS7-1093
Biotechnology / Synthetic Biology / Systems Biology - Part III

EXPRESSION OF GDSL ESTERASE FROM PSYCHROTOLERANT PSEUDOMONAS SP. S9 ISOLATED FROM SPITSBERGEN ISLAND SOIL IN PICHIA PASTORIS

M. Wicka-Grochocka[1], *E. Krajewska*[1], *H. Cieśliński*[1], *M. Wanarska*[1]
[1]*Gdansk University of Technology, Molecular of Biotechnology and Microbiology, Gdansk, Poland*

Backgrounds

Lipolytic enzymes isolated from cold adapted microorganisms are promising to replace the lipolytic enzymes recently used in biotechnological processes. One of the most important feature of the cold-active lipases and esterases is that they offer economic benefits through energy saving. Novel esterolytic enzyme was isolated from psychrotolerant *Pseudomonas* sp. S9. The enzyme is tolerant to alkaline pH and effective at medium to low temperatures (40-25°C). Amino acid sequence analysis revealed that the enzyme contained a G-D-S-L motif centered at a catalytic serine, an N-terminal catalytic domain and a C-terminal autotransporter domain.

Objectives

In our previous study, the genes estS9 and estS9auto was expressed in *E. coli* cells as His-tagged fusion proteins. Like many other recombinant proteins, EstS9Auto (variant which consist of two domains) and EstS9 (variant containing only the catalytic domain) were produced in *E. coli* in inclusion bodies. For these reason, we decided to check usefulness of other expression host for obtaining the extracellular active esterases. During this study we constructed recombinant *P. pastoris* strains for production of EstS9Auto and EstS9 proteins. The genes estS9 and estS9auto were expressed in the yeast *Pichia pastoris* under the control of alcohol oxidase (*AOX1*) promoter.

Methods

Methods included PCR, cloning of DNA fragments, transformation and gene expression in *P. pichia* system.

Conclusions

The EstS9Auto enzyme was effectively produced, but it was bound to yeast cell wall. However, EstS9 enzyme was produced to the culture medium. The biotechnological potential of new production system of EstS9 enzyme is tested at now.

FEMS7-0382
Biotechnology / Synthetic Biology / Systems Biology - Part III

NOVEL ACTIVITIES OF THE IRON–SULFUR PROTEIN ISPH AND ITS POTENTIAL IN PRODUCTION OF ISOPRENE AND ISOAMYLENE

Y. Xue[1], D. Ge[2], Y. Ma[1]
[1]Institute of Microbiology- CAS, Microbial Resources, Beijing, China
[2]Anhui University of Science and Technology, College of Medicine, Huainan, China

Backgrounds

Isoprene is an important chemicals and can be produced naturally by a wide variety of organisms, including animals, plants, and bacteria. *Bacillus* species, possessing the methylerythritol phosphate (MEP) pathway for the synthesis of isoprenoid feedstock, are the highest producers of isoprene among bacteria; however, the enzyme responsible for isoprene synthesis has not been identified.

Objectives

The iron–sulfur protein IspH is the final enzyme of the MEP pathway and catalyses the reductive dehydration of (E)-4-hydroxy-3-methyl-2-butenyl diphosphate (HMBPP) to form isopentenyl diphosphate and dimethylallyl diphosphate (DMAPP). In this study, we demonstrated two unexpected activities of IspH from alkaliphilic *Bacillus* sp. N16-5.

Methods

GC-MS analysis showed that IspH could catalyse the formation of isoprene from HMBPP and the conversion of DMAPP into isoamylene *in vitro* in the reaction system including IspH, NADPH, ferredoxin and ferredoxin-$NADP^+$-reductase. By site-directed mutagenesis, two variants (H131N and E133Q) were found to have lost the HMBPP reductase activity but could still catalyse the formation of isoprene. Overexpression of IspH H131N in *Bacillus* sp. N16-5 resulted in a twofold enhancement of isoprene production, and the yield of isoprene from the strain expressing E133Q was increased 300% compared with the wild-type strain.

Conclusions

IspH from *Bacillus* sp. N16-5 is a multifunctional enzyme that is responsible for isoprene and isoamylene synthesis in *Bacillus* sp. N16-5. The H131N and E133Q variants could be used for isoprene production from HMBPP, which may avoid the accumulation of prenyl diphosphate in the host cell.

FEMS7-0135
Biotechnology / Synthetic Biology / Systems Biology - Part III

PURIFICATION AND SOME PROPERTIES OF A THERMOSTABLE B-1,3-GLUCANASE FROM THERMOPHILIC ACTINOMYCETES

C.H. Yang[1], H. Jo-Chieh[1], C. Cheng-Yu[1], C. Wei-Lin[1], H. Yu-Chun[1]
[1]Providence University, Department of Cosmetic Science, Taichung, Taiwan R.O.C.

Backgrounds

Beta-1,3-gluans are complex biopolymers mainly found in mushroom yeast cell wall. Beta-1,3-gluanases can break down β-1,3-glucans such as callose or curdlan. To produce enzymes for the development of enzymatic degradation of the natural biopolymers, we isolated a potent extracellular hydrolases-producing thermophilic actinomycete, *Thermobifida fusca* BCRC 19214, from compost soils collected in Taiwan.

Objectives

The present study is concerned with the purification and properties of extracellular beta-1,3-glucanase from *T. fusca* BCRC 19214.

Methods

A beta-1,3-glucanase from *Thermobifida fusca* BCRC 19214 was purified from crude culture filtrate by DEAE-Sepharose CL-6B and Sepharose CL-6B column chromatography. The beta-1,3-glucanase activity was assayed with laminarin as a substrate. One unit of enzyme activity was defined as the amount of enzyme releasing 1 mmole glucose per minute under the assay condition. The amount of reducing sugar released in the mixture was determined by the dinitrosalicylic acid method.

Conclusions

The overall yield of the purified enzyme was 6.0%. The purified enzyme gave an apparent single protein band on an SDS-PAGE. The molecular mass of purified enzyme as estimated by SDS-PAGE was found to be 60 kDa. The optimum pH and temperature for the purified enzyme were 8.0 and 60^{o}C, respectively. The Zn^{2+}, Hg^{2+} inhibited the enzyme activity.

FEMS7-0518
Biotechnology / Synthetic Biology / Systems Biology - Part III

SIMULTANEOUS EXPRESSION OF THE POLYPHENOL OXIDASE AND XYLANASE GENE FROM THERMOPHILIC ACTINOMYCETES IN YEAST EXPRESSION SYSTEM

C.H. Yang[1], Y.C. Huang[1], Y.S. Lin[1], C.Y. Chen[1], W.L. Chen[1]
[1]Providence University, Department of Cosmetic Science, Taichung, Taiwan R.O.C.

Backgrounds

Thermobifida fusca is a thermophilic actinomycetes. It has the abilities to degrade plant biomass and to catalyze the oxidation of phenolic compounds. The polyphenol oxidase gene (*Tfu*-0957) and xylanase gen (*Tfu-xyl*) were cloned from *T. fusca* and expressed in *Escherichia coli* BL21 (DE3).

Objectives

The present study is concerned with the simultaneous expression of the polyphenol oxidase and xylanase gene from *T. fusc*a in *Pichia pastoris* expression system.

Methods

The *Tfu*-0957 and *Tfu-xyl* gene were cloned into pPICZαA and pPGAPZαA expression vectors, respectively, and then transformed into *P. pastori*s X-33 by electrophoresis.

Conclusions

The *P. pastoris* transformant selected by culture plates containing Zeocin and Geneticin could express two recombinant proteins. Xylanase was expressed constitutively and polyphenol oxidase was expressed inducibly. After cultivation in YPD medium for 5 days, there were 0.65 U/ml of polyphenol oxidase and 20.9 U/ml xylanase activities accumulated in the culture broth.

FEMS7-0423
Biotechnology / Synthetic Biology / Systems Biology - Part III

ENANTIOSELECTIVE SYNTHESIS OF (R)-PHENYLEPHRINE BY SERRATIA MARCESCENS BCRC10948 CELLS THAT HOMOLOGOUSLY EXPRESS SM_SDR

M.T. Yang[1], Y.C. Kuan[1], Y.B. Xu[1], W.C. Wang[2]
[1]National Chung Hsing University, Molecular Biology, Taichung, Taiwan R.O.C.
[2]National Tsing Hua University, Institute of Molecular and Cellular Biology, Hsinchu, Taiwan R.O.C.

Backgrounds

Optically active phenylephrine (PE) plays a rapidly growing role as a building blocks for the synthesis of pharmaceuticals and fine chemicals. Compared to chemical synthesis, the enzymatic synthesis of the chiral compounds is highly enantioselective and eco-friendly. To convert 1-(3-hydroxyphenyl)-2-(methylamino) ethanone (HPMAE) to optically pure (*R*)-PE, an NADPH-preferred short-chain alcohol dehydrogenase/reductase from *Serratia marcescens* BCRC10948, SM_SDR, had been cloned and expressed in *Escherichia coli*. However, a low conversion yield and productivity limited the application of this method in industrial processes.

Objectives

To produce optically pure (*R*)-PE from HPMAE with improved bioconversion efficiency, yield and productivity.

Methods

The homologous expression of SM_SDR in its native host *S. marcescens* BCRC10948 was carried out to produce optically pure (*R*)-PE from HPMAE with improved bio-catalytic efficiency. The cloned *SM_SDR* was driven by the T5 promoter and whole-cell bioconversion was applied to efficiently replenish the cofactor NADPH in the biocatalytic process. Glycerol was found to be the most effective carbon source for conversion of HPMAE to (*R*)-PE, and 6.50 mM of (*R*)-PE with more than 99% enantiomeric excess was produced from 10 mM HPMAE after a 10 h conversion at 30°C by the recombinant *S. marcescens* cells. The recombinant *S. marcescens* cells could be recycled 6 times for the production of (*R*)-PE, and the bioconversion efficiency remained at 85% when compared to that at the first cycle.

Conclusions

Our data indicated that a high conversion efficiency of HPMAE to optically pure (*R*)-PE could be achieved by using *S. marcescens* BCRC10948 cells that homologously express the *SM_SDR*.

FEMS7-1147
Biotechnology / Synthetic Biology / Systems Biology - Part III

GLYCEROL TRANSPORTER 1 INVOLVED IN THE REPRESSION OF GLYCEROL ON AOX1 EXPRESSION IN PICHIA PASTORIS

Y. Yang[1], *C. Zhan*[1], *Z. Bai*[1]
[1]*Jiangnan University, National Engineering Laboratory for Cereal Fermentation Technology, Wuxi, China*

Backgrounds

The P_{AOX1} (promoter of *alcohol oxidase 1* (*aox1*)) is the most efficient promoter used for promoting recombinant protein expression in *P. pastoris.* It is repressed by the presence of glycerol in the culture medium, thus glycerol must be exhausted before methanol can be taken up by *P. pastoris,* and subsequently the expression of heterologous protein is induced.

Objectives

Whether glycerol transporter exists and involves in the regulation of Aox1 expression or not?

Methods

A candidate GT1 (glycerol transporter 1) was identified by bioinformatics. It has been shown that GT1 is a transmembrane protein, and *Schizosaccharomyces pombe* (*S. pombe*) carrying *gt1* could grow on a medium containing glycerol as the sole carbon source while the wild type could not. The relationship between *aox1*, methanol expression regulator 1 (*mxr1*) and *gt1* was studied. It was found that the overexpression of *gt1* could increase the glycerol content in cells and repress the expression of *mxr1* and *aox1*, and the deletion of *gt1* reduced the glycerol content in cells and promoted the expression of *aox1*. The overexpression of *mxr1* could repress the expression of *gt1*, and the deletion of *mxr1* could promote the expression of *gt1* to some extent. By EMSA, Mxr1 could regulate the expression of *gt1* by binding to P_{Gt1}.

Conclusions

The Gt1 is the glycerol transporter and represses Mxr1 and Aox1 through increasing the glycerol content in cells. Mxr1 represses Gt1 through binding to $P_{Gt1,}$ which provides a reference for the understanding of the mechanism of glycerol repression on P_{AOX1}.

FEMS7-1971
Biotechnology / Synthetic Biology / Systems Biology - Part III

SCREENING AND IDENTIFICATION OF CHITINOLYTIC ENZYME FROM NATIVE HALOPHILIC ARCHAEA

M. yavari bafghi[1], *M.A. amoozegar*[1]
[1]*Faculty of Biology - College of Science - University of Tehran -, microbiology, Tehran, Iran*

Backgrounds

Chitin is the second most abundant natural polysaccharide. Chitinases are glycoside hydrolases catalyzing the hydrolytic degradation of chitin and have a wide range of applications including preparation of bioactive oligosaccharides, production of single cell proteins, pathogen inhibition and increasing the immune system of plants. Halophilic microorganisms are expected to produce extremozymes which are tolerable in high salt concentration and low water activity. These features are crucial in harsh conditions of industrial enzyme production.

Objectives

The aim of this study was to screen the chitinolytic enzyme produced by haloarchaea isolated from different saline and hypersaline lakes and wetlands in Iran, applying colloidal chitin as a sole source of carbon in culture media.

Methods

A total of 100 halophilic strains were selected for this project. Modified Growth Medium (MGM) supplemented with 1% colloidal chitin was used for qualitative assay of chitinase. After incubation at 40˚C and spending appropriate time, chitinolytic activity was visualized by a clear zone around colonies.

Conclusions

Among 100 strains, 3 strains were chitinase positive. Phylogenetic studies showed that these strains belonged to genera: *Haloarchaeobius*, *Halorubrum* and *Haloarcula*. This research showed that halophilic archaea from different saline environments in Iran have a potential of producing hydrolytic enzymes which may possess commercial value. Our further investigations will measure the enzyme activity and optimize the enzyme production by potent archea.

FEMS7-0751
Biotechnology / Synthetic Biology / Systems Biology - Part III

GENOME-WIDE IDENTIFICATION OF GENES INVOLVED IN NADPH SUPPLY

L. Ye[1], S. Cardinale[1], S. Siedler[1], M.M.H. Ellabaan[1], S.H. Kim[1], M.O.A. Sommer[1]
[1]Technical University of Denmark, DTU Biosustain, Kgs. Lyngby, Denmark

Backgrounds

NADPH is a crucial cofactor in reductive enzymatic reactions to synthesize valuable compounds. Previous designs to increase intracellular NADPH availability mainly focused on the modification of central metabolism. However, this may cause unexpected growth defect, such as the deletion of phosphoglucose isomerase (*pgi*) gene would dramatically slow down the growth rate compares to wildtype, letting alone the redirection of glycolysis flux significantly reduces the substrates level for the biosynthesis of most valuable chemicals.

Objectives

To identify mutants with both high NADPH supply and growth fitness.

Methods

We combined the fast fitness quantification by random barcoded transposon library (RB-TnSeq) and fluorescence-activated cell sorting (FACS) based on a NADPH biosensor. The mutant library (approximately 9×10^5 mutants each with unique useful barcode distributed in 3,882 genes) was constructed and characterized, and then transformed with plasmids containing the soxR NADPH biosensor. Libraries were selected in NADPH consuming condition, fitness/fluorescence change between NADPH depletion and control condition allows us to profile the intracellular NADPH level of each mutant. Thirty mutants were selected for better performance in NADPH consuming condition. These mutants were confirmed to have increased tolerance in NADPH depletion condition due to higher NADPH/$NADP^+$ ratio. They were further proved to have different levels of improvement for isobutyric acid and mevalonate production, both titer and yield compared to wildtype.

Conclusions

We established a high-throughput phenotype screening platform for *E. coli* K-12 strain. We proved the sufficiency to improve redox cofactor pool by eliminating unnecessary function genes, and extended the strain engineering space to genome-wide.

FEMS7-0328
Biotechnology / Synthetic Biology / Systems Biology - Part III

LACTOBACILLUS CASEI METABOLIZES THE GLYCOAMINOACID FUCOSYL-ALPHA-1,6-N-ACETYLGLUCOSAMINE-ASPARAGINE THAT FORMS PART OF HUMAN MILK AND MUCOSAL GLYCOPROTEINS

J.E. Becerra[1], J. Rodriguez-Diaz[2], M. Palomino-Schätzlein[3], M. Zuñiga[1], V. Monedero[1], M.J. Yebra[1]
[1]Instituto de Agroquímica y Tecnología de Alimentos, Biotecnología de Alimentos, Paterna, Spain
[2]Universidad de Valencia, Departamento de Microbiologia, Valencia, Spain
[3]Centro de Investigación Príncipe Felipe, RMN facility, Valencia, Spain

Backgrounds

Glycoproteins from human milk could play a significant role in the establishment of the infant gut microbiota. *N*-glycans contain an *N*-acetylglucosamine (GlcNAc) linked to asparagine and they can be modified with core alpha1-6-fucosylation. We have previously characterized the alpha-L-fucosidase AlfC encoded by *Lactobacillus casei* and analyzed the DNA region around *alfC*. This region (*alf2*) spans a hypothetical operon *alfHC*, encoding a permease and AlfC, respectively, and divergently oriented, a cluster of genes annotated as *asdA*, *alfR2*, *pepV*, *asnA2* and *sugK*, encoding hypothetical aspartate decarboxylase/aminotransferase, transcriptional regulator, peptidase, glicosylasparaginase and sugar kinase, respectively.

Objectives

1) To study the role and transcriptional regulation of the *alf2* operon from *L. casei*.

2) To synthesize 6'fucosyl oligosaccharides and fucosyl-alpha-1,6-*N*-GlcNAc-Asn (6'FN-Asn) using AlfC in transglycosylation reactions.

Methods

Construction of mutant strains by plasmid insertional inactivation of genes in *L. casei*; RNA isolation and RT-qPCR; transglycosylation reactions with purified 6X(His)AlfC and *p*-nitrophenyl-alpha-L-fucopyranoside as donor; HPLC analysis; RMN.

Conclusions

1) 6'FN-Asn, fucosyl-alpha1,6-glucose, fucosyl-galactose and fucosyl-*N*,*N*'-diacetylchitobiose were synthesized by AlfC in transglycosylation reactions with maximum yields of 3.6, 3.3, 1.3 and 1.8 g/l, respectively.

2) *L. casei* is able to grow in the presence of the glycoaminoacid 6'FN-Asn, and the permease AlfH and the alpha-L-fucosidase AlfC, encoded in the *alf2* operon, are involved in its metabolism.

3) The expression of *alf2* operon is induced by 6'FN-Asn through the AlfR2 transcriptional repressor. Unlike the wild-type, an *alfR2* mutant strain can metabolize the disaccharides fucosyl-alpha-1,6-GlcNAc, fucosyl-alpha1,6-glucose and fucosyl-galactose, and the trisaccharide fucosyl-*N*,*N*'-diacetylchitobiose.

FEMS7-1156
Biotechnology / Synthetic Biology / Systems Biology - Part III

PRODUCTION OF LACCASE AND CELLULASES BY PYCNOPORUS SANGUINEUS (DMSZ 3024) USING HAZELNUT HUSK

Z. Yılmaz Serçinoğlu[1], N.A. Sayar[1], D. Kazan[1]
[1]Marmara University, Bioengineering, İstanbul, Turkey

Backgrounds

The cost of enzyme cocktails used for the pretreatment and hydrolysis of lignocellulosic raw material is one of the main obstacles to the successful commercialization of second-generation bioethanol. Integrated production of these enzymes and the subsequent bioethanol production may be an approach to lower overall production costs. Using the lignocellulosic raw material as substrate for the production of the lignocellulolytic enzymes may help simplify the integration of enzyme and bioethanol production processes. Hazelnut husk is an abundant agricultural waste in Turkey which can be used as lignocellulosic raw material. It is comparably lignin rich and its biological pretreatment with laccase to enhance delignification is important for later efficient hydrolysis. So, the production of an enzyme cocktail containing laccase and cellulase enzymes using hazelnut husk as substrate may be advantageous for later integration with bioethanol production.

Pycnoporus sanguineus DMSZ 3024 (*P.sanguineus*) is a white rot fungus which is a potential producer of an enzyme cocktail containing laccase and cellulase enzymes.

Objectives

Our aim is to identify and assess the potentiality of *P. sanguineus* as a producer of the lignocellulolytic enzyme cocktail using hazelnut husk as substrate.

Methods

Laccase, CMCase, β-glucosidase are produced by *P. sanguineus*. Activity of lignocellulolytic enzymes are measured daily by using CMC, pNPG and ABTS as substrate, respectively.

Conclusions

It is found that *P. sanguineus* produces the lignocellulolytic enzyme cocktail containing laccase, CMCase, β-glucosidase and is open to further optimization and integration with bioethanol production processes.

FEMS7-1312
Biotechnology / Synthetic Biology / Systems Biology - Part III

MICROALGAE AS CELL FACTORIES: SYNTHETIC TRANSFER RNA GENES AS GENETIC ENGINEERING TOOLS FOR THE CHLAMYDOMONAS REINHARDTII CHLOROPLAST

R. Young[1], *S. Purton*[1]
[1]*University College London, Structural and Molecular Biology- Darwin Building, London, United Kingdom*

Backgrounds

Single-celled eukaryotic microalgae such as *Chlamydomonas reinhardtii* have potential as an alternative platform for the production of vaccines, antibiotics and a variety of chemicals. Benefits include their ability to fix carbon using sunlight, ease of scaleup, lack of endotoxins and the availability of genetic engineering tools.

Objectives

We previously developed a chloroplast transfer RNA (tRNA) that allows the constitutive translation of UGA codons as tryptophan; UGA is not naturally used as either a sense or stop codon in the *C. reinhardtii* chloroplast. This tool has applications for cloning antibacterial genes and biocontainment. We now aim to address the pressing need for a simple inducible system for the expression of foreign proteins and metabolic pathways in the microalgal chloroplast.

Methods

We are working to identify temperature-sensitive variants of our original trnW-UCA tRNA in order to develop a cold-inducible expression system for transgenes with internal TGG-to-TGA alterations. One variant in particular is displaying promising thermolabile behaviour and we are characterising this further.

Conclusions

A temperature-inducible protein expression system would avoid the need for an expensive chemical inducer and could be used in any *C. reinhardtii* strain background. We hope to adapt the stable and thermolabile versions of trnW-UCA for other industrially relevant microalgae such as *Phaeodactylum* and *Chlorella*.

FEMS7-1412
Education / Professional Development / Policies

THE EDUCATION AND COMMUNICATION DIVISION OF THE SPANISH SOCIETY FOR MICROBIOLOGY (D+D SEM)

I. Arana[1,2], I. López-Goñi[2,3], D. Vidal[2,4], I. Belda[2,5], V. Jiménez Cid[2,6], M.F. Colom[2,7], M. Llagostera[2,8], M. Martínez-Cañamero[2,9], M. Sánchez[2,7], M.J. Valderrama[2,5], A. Ventosa[10,11]
[1]Universidad del País Vasco UPV/EHU, Inmunología- Microbiología y Parasitología, Leioa, Spain
[2]Spanish Society for Microbiology Sociedad Española de Microbiología- SEM,
The Education and Communication Division Docencia y Difusión- D+D SEM, Madrid, Spain
[3]Universidad de Navarra, Microbiología y Parasitología, Pamploa, Spain
[4]Universidad de Castilla-La Mancha., Ciencias Médicas, Ciudad Real, Spain
[5]Universidad Complutense de Madrid, Microbiología III, Madrid, Spain
[6]Universidad Complutense de Madrid, Microbiología II, Madrid, Spain
[7]Universidad Miguel Hernández, Producción Vegetal y Microbiología, Orihuela, Spain
[8]Universitat Autònoma de Barcelona, Genètica i Microbiologia, Bellaterra Cerdanyola del Vallès, Spain
[9]Universidad de Jaén, Ciencias de la Salud, Jaén, Spain
[10]Universidad de Sevilla, Microbiología y Parasitología, Sevilla, Spain
[11]Spanish Society for Microbiology Sociedad Española de Microbiología, SEM, Madrid, Spain

Backgrounds

The Education and Communication Division (Docencia y Difusión, D+D SEM; www.semicrobiologia.org/ddm) of the Spanish Society for Microbiology (Sociedad Española de Microbiología, SEM; www.semicrobiologia.org) was created as a transversal group in which researchers, teachers, journalists and others can collaborate to promote quality and veracity in Microbiology transmission.

Objectives

To improve the microbiological knowledge of the society and to promote the elaboration and open exchange of materials and resources for Microbiology Education.

Methods

University and Research Centers' staff who teach or investigate in diverse Microbiology areas all along Spain are members of the Division, ensuring a diversity of knowledge and interests.

D+D SEM searches to establish links with professionals at primary and secondary schools, offering assessment and support about microbiological subjects, aiming to the content of official teaching modules and text books and editing a book of educative short stories aimed to children. Moreover, members publish microbiological concepts by participating in scientific fairs, advising journalists and by the use of the internet and social networks (official and personal blogs and networks).

D+D SEM organizes yearly workshops on "Initiation to Research in Microbiology" for brilliant students and hosts the Group of Young Researchers (sites.google.com/site/jovenesinvestigadoressem).

Periodically, D+D SEM organizes congresses and meetings so that its members and any teacher or researcher interested can share experiences and knowledge and also promotes the participation in any event of interest.

Conclusions

We seek to share our experience and to coordinate our effort with similar initiatives in other countries.

FEMS7-1587
Education / Professional Development / Policies

ACTIVITIES OF THE YOUNG RESEARCHERS GROUP OF THE SPANISH SOCIETY FOR MICROBIOLOGY

I. Belda[1], S. Bárcena[2], J.D. Flores-Felix[3], D. Thomas-Lopez[4], B. Vera-Gargallo[5]
[1]Biology Faculty- Complutense University of Madrid, Department of Microbiology, Madrid, Spain
[2]University of Navarra, Department of Microbiology and Parasitology, Pamplona, Spain
[3]University of Salamanca, Department of Microbiology and Genetics, Salamanca, Spain
[4]Faculty of Veterinary Medicine and VISAVET- Complutense University of Madrid, Animal Healh Department, Madrid, Spain
[5]Faculty of Pharmacy- University of Sevilla, Department of Microbiology and Parasitology, Sevilla, Spain

Backgrounds

The current socio-economic situation has led to young scientists facing a harsher, more competitive, more dispiriting workplace than previously. In this context, Scientific Societies must promote and facilitate scientific careers by boosting motivated and vocational researchers from undergraduate levels and improving later scientific networks.

Objectives

Since 2012, a group of PhD students have developed the Young Researchers Group (JISEM) within the Spanish Society for Microbiology (SEM). Our goal is to increase the bonds of young Spanish microbiologists with our Society-SEM, by improving their “scientific experience” during their PhD and first postdoc stages.

Methods

We have established different work fronts:

- Communicating and sharing research opportunities

- Introducing young researchers in the Boards of Trustees of different SEM Committees

- Contributing to the SEM annual course “Research in Microbiology”

- Promoting the participation of SEM-granted students in National Meetings

- Writing outreach articles in SEM-Newsletter about “Success stories” of Spanish and International young microbiologists

- Developing census and statistics about Spanish microbiologists

Conclusions

66 students received a SEM Fellowship for the “Research in Microbiology” course and to attend SEM National Congresses. 48 of those are now young members of SEM starting their PhDs projects.

We have a *junior* representative in 3 SEM-Scientific Committees now, and we expect this number to increase during the next years.

We have organized 2 successful “Young researchers” discussion forums at SEM Conferences and written 27 articles regarding worldwide initiatives and microbiologists. Our active Facebook group shares scientific information, with more than 1500 interactions per week.

FEMS7-1209
Education / Professional Development / Policies

A LABORATORY ACTIVITY USING BACTERIOPHAGES, THE FORGOTTEN WEAPON AGAINST BACTERIA

S. Campoy[1], C. Pilar[1], J. Aranda[1], M. Sánchez-Osuna[1], J. Barbe[1], M. Llagostera[1]
[1]Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, Cerdanyola del Vallès, Spain

Backgrounds

Soon after their discovery, 100 years ago, phages were used to treat patients suffering from dysentery or cholera and to promote wound recovery. Although the results were promising, interest in phage therapy waned until it was mostly forgotten, as penicillin and other antibiotics became widely available. Today, the emergence of multidrug-resistant bacteria has rekindled the interest in bacteriophages as antibacterial tools. Despite phages are the most divers and abundant entities in our planet and their multiple applications, biology students remain largely unaware of these entities and are offered few opportunities to explore them in the laboratory.

Objectives

In this activity, students are introduced to the discovery of bacteriophages and encouraged to design an experiment demonstrating the possible use of lytic phages as biocontrol agents and comparing their efficacy with that of other antimicrobial agents. Further, they also formed hypotheses about the expected results and then, using quantitative data, tested their validity.

Methods

Experimental data were obtained by measuring absorbance and cell viability of *Salmonella enterica* sv Typhimurium LT2 cultures treated with either the P22 lytic derivative bacteriophage or different antibiotics (streptomycin, ampicillin or spectinomycin).

Conclusions

The obtained data, combined with class data or student autonomous learning, contributed to providing insights into the lytic and lysogenic cycles of bacteriophages, the molecular processes that finally kill the bacteria, and the appearance resistant bacteria. Further, using the scientific method, students were able to explore the nature of antimicrobial agents and the possible use of phages, phage cocktails, and phage derivative compounds as antibacterial tools.

FEMS7-1875
Education / Professional Development / Policies

CONTINUOUS ASSESSMENT IN MICROBIOLOGY SUBJECTS IMPROVES ACADEMIC PERFORMANCE IN UNDERGRADUATE MEDICAL STUDENTS

M.F. Colom[1]
[1]*Universidad Miguel Hernández, Producción Vegetal y Microbiología, Sant Joan d'Alacant, Spain*

Backgrounds

The influence of the method of assessment, regardless the teaching system, is always critical in training the students and in the whole teaching process.

During the Bologna process we had the chance to review our teaching and training methods in higher education. This analysis together with the results obtained in local, specific surveys carried out in our faculty of medicine, were the basis for a major modification in the teaching methodology in Microbiology subjects for medical students.

Objectives

Assessment of the learning process based on continuous assessment methodology for teaching microbiology to medical students

Methods

After 10 academic years using continuous assessment methodology in teaching microbiology to medical students, we assess the assessment method through the analysis of three parameters: final grades, students' direct answers in quality surveys and the teacher’s grades in the university official assessment of the teaching system.

Conclusions

Analysis of the grades obtained in the ordinary call (February or June), show a significative increase in the mean (from 6.1 to 6.7) and the mode values (from 5.4 to 6.4) of final grades and in the percentage of students who passed the subject (from 61.8 to 96.2%). Similar results were obtained globally in teachers' assessments and the teaching quality surveys, with a positive increase of the qualitative and quantitative parameters evaluated.
The evaluation of three parameters related with the quality of the teaching process evidenced that continuous assessment method improve the performance of undergraduate medical students

FEMS7-0601
Education / Professional Development / Policies

SIMPLE PROTOCOL FOR MOLECULAR FINGERPRINTING OF HUMAN ORAL MICROBIOTA SAMPLES IN LAB CLASSES

A.C. Henriques[1], P. De Marco[1]
[1]IUCS, IINFACTS, Paredes, Portugal

Backgrounds

The increasing significance of molecular biology in different circumstances of our daily life implies that it is very important that biomedical students deal with the principles and techniques underpinning its application. Human DNA fingerprinting is a major tool in identifying individuals and in evidence matching. However, this technique can be difficult to reproduce in practical classes.

Objectives

Create a practical class activity suitable for undergraduate students that elucidates how human oral microbiota is diverse and individually unique.

Methods

Here we report on distinct PCR profiles obtained when amplifying saliva DNA of a score of distinct individuals with RAPD primer BOXA1R. RAPD is a simple method efficiently used for discrimination between bacterial strains and is used in this instance to obtain personalized fingerprints of each individual's oral microbiota.

Conclusions

We present real results with undergraduate students confirming that this procedure is easily feasible in practical classes. Based on the results presented, we suggest a laboratory activity for undergraduate Molecular Biology / Microbiology students.

FEMS7-0754
Education / Professional Development / Policies

GETTING INTO THE SWING OF THINGS - COMMITTING TO LIFELONG LEARNING AND DEVELOPMENT (FROM UNDERGRADUATES TO ACADEMICS)

B. Fahnert[1]
[1]*Cardiff University, Cardiff School of Biosciences, Cardiff, United Kingdom*

Backgrounds

In a rapidly changing world, where jobs are no longer for life or change beyond recognition, lifelong learning is required of everyone. Educators need to support learners early on in understanding this need and developing necessary skills and reflection. So initial Personal Development Planning prepares and becomes Continuous Professional Development. Equally, these educators need sufficient opportunity for pedagogic training, also perceived as valuable by undergraduates and benefitting academic career establishment/advancement. Teaching professionalism is not developed through traditional academic apprenticeship (i.e. doctoral studies), and -along with high graduate employability rates- has become a focus for Higher Education Institutions to boost institutional reputation.

Objectives

Better understand behaviour and drivers around committing to lifelong learning at two stages:

a) graduate attributes and career skills development
to inform practice when equipping/empowering undergraduates for successful transition into employment;
b) teaching training for early career academics
to achieve long-term benefit for all stakeholders.

Methods

Stakeholder and discourse analyses and ethically cleared mixed method studies (interviews, questionnaires) were conducted, qualitatively analysing experiences and perceptions of:

a) under/postgraduates, academics, employers, alumni;

b) teaching training participants and non-participating academics.

Conclusions

In both contexts behaviour is affected by conflicting demands on time at present and obtaining intangible benefits for an unknown future.

Undergraduates prioritise subject rather than career skills unless incentivised, which still does not guarantee meaningful engagement.

Challenges to teaching training were e.g. non-peer trainers, unfamiliarity with terminology and using qualitative methodology/findings.

Using identified drivers to tailor developmental provision and communication with stakeholders towards cultural change is outlined.

FEMS7-2205
Education / Professional Development / Policies

UNDERGRADUATE EDUCATION IN MICROBIOLOGY - A MULTIPLE APPROACH IN UNIVERSITY OF PORTO

R. Mota[1], J. Palmeira[2], D. Gonçalves[3], H. Ferreira[2]
[1]University of Porto, Microbiology - Pharmacy Faculty, Porto, Portugal
[2]University of Porto, Microbiology - Pharmacy Faculty - UCIBIO, Porto, Portugal
[3]University of Porto, Microbiology - Pharamacy Faculty - UCIBIO, Porto, Portugal

Backgrounds

University of Porto has implemented a program of stimulation of Science Education of secondary school students emphasizing Microbiology. Community joined this approach particularly Municipality of Porto in cooperation with University and Research Institutes.

Objectives

Enhance scientific literacy in Microbiology and interest of the youngsters for science and health related experimental areas.

Methods

Junior University is a Summer School program including Microbiology related programs as "Microbiology an experimental approach" that develops skills in laboratory research in Microbiology. Another program intended for older excellent secondary school students, School of Sciences of Life and Health as for example "Colonization and infection two faces of the coin" and "Antimicrobial Resistance an ecological perspective".

Junior Research at University of Porto is another approach in which undergraduate students participate in research in different faculties with a great impact in Microbiology studies.

The interest of these approaches is shown by the dynamism of participation in National Science divulgation events as Young Scientists Annual Competition, private foundation awards and University of Porto Young Researchers Congress.

Conclusions

Junior University turned into an ex-libris of University of Porto and every year Junior University program has a very competitive choice by thousands of students of all over the Country. Microbiology is one of the most wanted areas. This approach is relevant in terms of areas and courses that young students will choose for University. This engages community in public presentations of works at the end of the program in a true scientific congress. There are enthusiastic results for the long term impact of these programs.

FEMS7-0678
Education / Professional Development / Policies

SOCIAL NETWORKS AS KEY TOOLS IN MICROBIOLOGY DIVULGATION

J.D. Flores Felix[1], P. García-Fraile[2], P. Martínez-Hidalgo[1], M. Marcos-García[1], L. Celador-Lera[1], A. Díez-Méndez[1], X.A. Cruz-González[1], A. Jimenez-Gomez[1], R. Rivas[1]
[1]University of Salamanca, Microbiología y Genética, Salamanca, Spain
[2]Academy of Science of the Czech Republic, Department of Microbiology, Prague, Czech Republic

Backgrounds

YouTube is the major online video platform, with a high diversity of contents and over a billion of users from all around the world. Therefore, this platform has a high potential as education and divulgation tool. The combination of this platform with the social networks, such as Facebook or Twitter, has a great potential to introduce academic contents to a non-academic audience

Objectives

Our aim was to use of social networks as a tool to improve the diffusion of the Microbiology using multimedia contents guested in YouTube, increasing the number of potential spectators reached by our videos about microbiology techniques hosted in of YouTube channel.

Methods

We created different profiles in YouTube, Facebook and Twitter. The Youtube profile was linked to own channel, which has with 215 subscriptions and counts more than 30.000 video visualizations. On Facebook, we opened a Facebok Page named “Microbiología y Genética. Interacciones Planta-Microorganismo. USAL” (translated into English: Microbiology and Genetics. Plant-Microbes Interactions. USAL”) and on Twitter we created the profile @MicrobioUSAL. Then, we shared our Microbiology YouTube videos on our social networks.

Conclusions

We have seen than YouTube is a useful tool to disseminate microbiology contents into academic and non-academic fields and moreover, the use of social networks such as Facebook and Twitter to share the videos hosted on YouTube allowed us to increase the number of spectators, proved by peaks in the numbers of visualizations and minutes with clear activity during few hours following their shares on the social networks.

FEMS7-2231
Education / Professional Development / Policies

IMPACT OF SWI@SPAIN PROJECT IN DIFFERENT EDUCATIONAL AND SOCIAL CONTEXT HIGH SCHOOLS FROM THREE AREAS OF COMUNIDAD DE MADRID

C. Vázquez[1], J. Gil-Serna[1], M. García-Díaz[1], C. Briceño[2], T. Patiño[3], A. Zorrilla[4], A. García-López[1], C. Martín[1], A. Martinez[1], R. Pedrero[1], G. Saiz[1], B. Salazar[1], P. San Segundo[1], J. Sempere[5], I. Veliz[1], B. Patiño[1]
[1]Universidad Complutense de Madrid, Facultad de Biologia- Microbiología III, Madrid, Spain
[2]IES El Escorial, Ciencias, El Escorial, Spain
[3]IES Calderón de la Barca, Ciencias, Madrid, Spain
[4]IES Margarita Salas, Ciencias, Majadahonda, Spain
[5]Universidad Complutense de Madrid, Facultad de Químicas, Madrid, Spain

Backgrounds

Antimicrobial resistance (AMR) is a global concern that affects all society. An effective prevention involves a coordinated action at all levels including the educational one. The university has always served as a motor in which knowledge is transmitted and transformed to benefit society. In this context, SWI@Spain (Small World Initiative at the Complutense University of Madrid, Spain) is an Educational Project that implements the American SWI program in Spain.

Objectives

To encourage high school students to pursue careers in science while addressing AMR by establishing the first steps of scientific investigation in this matter, under the supervision of SWITAs, SWIPIs and high school students tutors.

Methods

The Spanish SWI project units undergraduate and Education master students from the Biological Science Faculty, all supervised by a Microbiology professor. SWI protocols were used for establishing the experiments with International Baccalaureate students, Bilingual Baccalaureate students and Baccalaureate students who were 18+ years old. The tasks included: preparing teaching and lab materials as well as supervising the development of the experiment. In addition, we evaluated the pedagogical effects of implement this innovative initiative by conducting satisfaction surveys.

Conclusions

The results of the various surveys among high school students have reflected a positive impact on motivation and basic AMR knowledge. In addition there is a growing interest in general science interest and curiosity, highlighting how high school students aim their classmates to participate in the SWI project.

FEMS7-2343
Education / Professional Development / Policies

IMPACT OF SWI@SPAIN PROJECT IN SMALL WORLD INITIATIVE TEACHER ASSISTANTS (SWITAS)

J. Gil Serna[1], C. Vazquez[1], A. García-Lopez[1], C. Martin[1], A. Martinez[1], R. Pedrero[1], G. Saiz[1], B. Salazar[1], P. San Segundo[1], J. Sempere[2], I. Velez[1], B. Patiño[1]
[1]Complutense University, Microbiology III, Madrid, Spain
[2]Complutense University, Facultad de Quimicas, Madrid, Spain

Backgrounds

Antimicrobial resistance (AMR) is a global concern that affects all society. An effective prevention involves a coordinated action at all levels including the field of education. The university has always served as a motor in which knowledge is transmitted and transformed to benefit society. Undergraduate students are not accustomed to be in contact with teaching and real-life research experiences. SWI@Spain (Small World Initiative at the Complutense University of Madrid, Spain), which is supported by INNOVA- Docencia program, helps our students at this level.

Objectives

To offer STEM undergraduate students the possibility to expand their knowledge and open a wide variety of new job opportunities, while addressing AMR by doing all the main steps in scientific research under the supervision of SWIPIs.

Methods

SWITAs are undergraduate students from the Biology and Biochemistry degree who were selected by a Microbiology professor. These students were informed about the international Small World Initiative, as they received an intensive training to prepare them to being able to teach high school students the AMR issue and scientific method: highlighting biosecurity, tenacity and scientific accuracy. The pedagogical and scientific effects of this innovative initiative was evaluated among the students. They completed a-15 question survey, which include questions related to science, teaching and AMR aspects.

Conclusions

The result of the different surveys reflected a positive impact on motivation and general AMR knowledge, as well as an increment in investigation and teaching interest. Some of these students have joined various university investigation teams, improving their scientific knowledge.

FEMS7-0501
Education / Professional Development / Policies

"HOW ARE MICROBES USEFUL TO US?": MICROBIOLOGY LEARNING IN EUROPEAN RESEARCHER'S NIGHT.

A. Jiménez-Gómez[1], X. Cruz-González[1], J.D. Flores-Félix[1], L. Celador-Lera[1], A. Diéz-Mendez[1], E. Menéndez[2], M. Marcos-García[1], P. García-Fraile[3], R. Rivas[1]
[1]University of Salamanca, Department of Microbiology and Genetics, Salamanca, Spain
[2]Instituto de Ciências Agrárias e Ambientais Mediterrânicas, ICAAM, Evora, Portugal
[3]Academy of Science of Czech Republic., Institute of Microbiology, Prague, Czech Republic

Backgrounds

European Researcher's Night is one of the most important scientific events in Europe. It takes place every year on the last Friday in September. More than 30 countries and over 300 cities participate organizing scientific dissemination activities, showing to citizens what researchers really do for society and increasing the possibility to promote research careers to young people. In addition, Microbiology is one of the subjects which does not have an important relevance in the current high schools' curricula, and however has a big relevance in our lives. Moreover, microbiology requires from society the comprehension of many complicated concepts, which are easy to learn if taught in a practical way.

Objectives

In this context, our main objective was to offer society the possibility of seeing colonies of microorganisms cultured in Petri dishes, observing different samples under the microscope and showing the essential importance of microorganism in many key points of our daily life.

Methods

The activity was carried out in one of the microbiology laboratories of the University of Salamanca (Spain). The 40 participants were divided into small groups and were rotating to perform the different activities in one-hour sessions. The language used was suited to the different age of the participants (between 14 and 70 years old) and the topics presented were selected according to general society interests.

Conclusions

The experience was gratifying and positive not only for the participants but also for the researchers who organized the activity. It was an exchange of questions and answers which showed the general doubts of the society about Microbiology. The success of the activities shows us that the topics presented were interesting to both children and adults.

FEMS7-1845
Education / Professional Development / Policies

HOW DO BACTERIAL PATHOGENS SPREAD IN A COMMUNITY?

M. Llagostera[1], S. Campoy[1], J. Barbe[1], M. Marco[2], N. López[3], S. Lope[4]
[1]Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, Cerdanyola del Vallès, Spain
[2]Institut Sant Quirze del Vallès, Secció Biologia, Sant Quirze del Vallès, Spain
[3]Generalitat de Catalunya, CESIRE - àmbit de ciències CDEC- Servei d'Innovació i Formació de l'Educació Primària. Departament d'Ensenyament, Barcelona, Spain
[4]Generalitat de Catalunya, CESIRE - àmbit de ciències CDEC- Servei d'Innovació i Formació de l'Educació Secundària. Departament d'Ensenyament, Barcelona, Spain

Backgrounds

Numerous bacterial infections may be transmitted through direct or indirect contact with a reservoir of infectious bacteria. Direct person-to-person contagion occurs when an individual comes into contact with the reservoir via touching infected bodily fluid; sharing beverages containing bacteria or inhaling the pathogens contained in little water droplets, often emitted by sneezing or coughing or even by a person when speaks. This can happen before an infected person is aware of the illness. Here we present a laboratory activity designed by the µBIO Cat Group to introduce primary and secondary education students to the contagion of bacterial diseases.

Objectives

In this activity the students simulate the pathogen bacteria spread from an infected person to the rest of the community. The challenge is to identify the infection focus and to propose methods for the contagion prevention.

Methods

Rhodobacter sphaeroides, a non-pathogenic bacteria that give rise red colonies, was chosen as the bacterial agent to be spread and students shook their hands to promote the person-to-person contact. In order to identify the first infected person, samples from each student's hand were taken, plated onto suitable media and bacterial colonies were quantified.

Conclusions

This activity was performed in different educational centers. The experimental obtained results and the conclusions achieved in each case have been exposed and discussed. Further, the opinions and satisfaction levels of both, teachers and students, about the activity have been also collected and analyzed.

FEMS7-3117
Education / Professional Development / Policies

ESSENTIAL MICROBIOLOGY: THE FIRST GENERAL MICROBIOLOGY TEXT-BOOK WRITTEN BY SPANISH MICROBIOLOGISTS

A. Martin-Gonzalez[1], V. Bejar[2], J.C. Gutierrez[1], M. Llagostera[3], E. Quesada[2]
[1]Universidad Complutense UCM, Microbiologia-III Fac. Biologia, Madrid, Spain
[2]Universidad de Granada, Microbiologia. Fac. Farmacia, Granada, Spain
[3]Universidad Autonoma de Barcelona., Genetica y Microbiologia, Barcelona, Spain

Backgrounds

Four years ago, we embarked on an exciting adventure to elaborate the first Spanish textbook on General Microbiology. The aim was that this book could be used as a basic tool for teaching the microbial word to undergraduate students of very diverse University Degrees, such as Biology, Biochemistry, Biotechnology, Environmental Sciences, Microbiology, Pharmacy, etc. Fortunately, this adventure has become a reality and finally, the textbook, entitled *Essential Microbiology*, is already in press. It will be published by Panamericana Press (Editorial Panamericana) in 2018.

Objectives

The elaboration of the different book chapters has been carried out by 33 different authors, which are Spanish microbiologists, all members of diverse specialized groups of the *Spanish Society of Microbiology* (SEM). *Essential Microbiology* is organized in 38 chapters, distributed in seven thematic blocks (Introduction, Microbial Structure and Cell Differentiation, Metabolism and Growth, Microbial Genetics and Genomics, Microbial Diversity, Ecology and Microbial Interactions, and Microbial Biotechnology).

Methods

Some of these chapters are usually included in Microbiology textbooks. Nevertheless, in this book, new views have been incorporated, such as Immunology as an aspect of human-microbe interaction; it also contains a number of new chapters habitually not included in a traditional Microbiology book; for instance; microbial gene expression regulation, microbial genomics, sexual processes in eukaryotic microorganisms, eukaryotic microbial structure, viruses of eukaryotic microorganisms and plants, and interactions among microorganisms.

Conclusions

Likewise, all chapters include: illustrative schematic diagrams, sample and self-evaluation questions, short hot topics in Microbiology, a summary of the main concepts, basic references, a glossary, and other tools to facilitate the learning and curiosity of potential students. We hope that this book becomes an useful tool for both university students and professors, in a next future.

FEMS7-2399
Education / Professional Development / Policies

THE SMALL WORLD INITIATIVE AS A TOOL TO LEARN ABOUT ANTIFUNGAL DRUG DISCOVERY, TARGET-BASED SCREENING AND SYNTHETIC BIOLOGY

H. Martín[1], A. Alvarez[1], I. Bascones[1], P. Calomarde[1], T. Sardón[1], B. Ureña[1], A. García[2], T. Martin[2]
[1]Universidad Complutense de Madrid, Microbiología II, Madrid, Spain
[2]Colegio Mirabal, Bachillerato, Boadilla del Monte, Spain

Backgrounds

Introduced at Yale University in 2012, the Small World Initiative (SWI) is an educational program that encourages young students to pursue careers in science and to raise awareness about what is considered for WHO one of the main health threats: the scarcity of effective antibiotics. This is particularly for antifungal drugs since resistance to them has emerged as a therapeutic challenge.

Objectives

We wanted to extend the main objectives of the general SWI program to (i) the awareness of the antifungal drugs insufficiency, (ii) the knowledge about the use of target-directed screenings to search for antibiotics with a specific mode of action and (iii) the possibilities and concerns about the use of synthetic biology for reprogramming cell behavior.

Methods

A group of 5 Pharmacy students coursing Microbiology at the third year, supervised by a Faculty Microbiology professor and two high-school professors, implemented SWI on a group of 32 high-school students taking biology at the Mirabal School. A total of 16 soil samples were screened for the existence of bacteria able to produce antibacterial or antifungal molecules. Whereas classical bacterial strains were used for detecting antibacterial producing microorganisms, a novel yeast strain constructed by a synthetic biology approach was used to sense cell wall altering antifungal molecules.

Conclusions

As a service-learning project, this SWI project has been useful not only to search for novel antibiotics but also to help students to learn and think about the necessity of antifungal drug discovery programs and the utility of synthetic biology to this end.

FEMS7-1591
Education / Professional Development / Policies

HIGH SCHOOL STUDENTS FOR MICROBIOLOGICAL SCIENCE RESEARCH

F. Martinez-Abarca[1], *A. Quesada-Ramos*[2], *F.J.P. Caceres*[3]
[1]*Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas, Microbiología del Suelo y Sistemas Simbióticos, Granada, Spain*
[2]*IES Zaidin Vergeles, Departamento de Biologia y Geologia., Granada, Spain*
[3]*Universidad de Granada, Departamento de Didáctica de las Ciencias Experimentales, Granada, Spain*

Backgrounds

Innovative ideas are always welcomed to improve learning in educational environments. Innovation at High School in Andalucía (current acronym *Science-IES*; http://piiisaandalucia.blogspot.com.es/p/que-es-piiisa.html) was run last years as the collaboration among centers of the Consejo Superior de Investigaciones Científicas (CSIC) , Universities and Secondary Schools of Junta de Andalucía (Spain). *Science-IES* brought High School Students to laboratories of the Research Institutes to develop small research projects during 7-9 sessions throughout the academic course. Those projects allow the interrelationship among students, teachers and scientists to work “hand in hand” for the same objective. The microbiology discipline has been already present in 6 editions of this event (2012-).

Objectives

To design simple or not so simple experiments to introduce Microbiology concepts (gene, genome, mutation, mutagenic effects, …) to teenager students finishing in a written and oral comunications.

Methods

Students (15-16 years old) will become familiar with Molecular Biology concepts and basic techniques in Microbiology (dilutions, colony counts, DNA isolation, PCR, DNA sequencing, …) in order to acquire how research in Microbiology is conducted (Example in http://mutandogenes.blogspot.com.es/).

Conclusions

This model has created a new manner to built science, with very profitable results in laboratories, but, as the most prominent consequence, it has proposed a new way to understand how to delight/teach/learn microbiology (as well as other disciplines) at high schools. *Science-IES* shows to high school students how research is performed today.

Acknowledgements: FECYT-FCT-12-3552 and research-projects CSD2009-00006; BIO-2011-24401 and BIO2014-51953-P *from the Spanish Government all including European Regional Development Funds.* Also we appreciate the coordination of Secondary Schools teachers involved

FEMS7-1711
Education / Professional Development / Policies

STUDENT-GENERATED MULTIPLE-CHOICE PRE-EXAM QUESTIONS: AN EFFECTIVE TOOL FOR PARTICIPATORY LEARNING IN MICROBIOLOGY

M. Marvasi[1], M. Teplitski[2], M. Di Cesare[1], C.J. Krediet[3]
[1]Middlesex University, Natural Sciences, London, United Kingdom
[2]University of Florida, Water and Soil Science, Gainesville, USA
[3]Eckerd College, Marine Biology Department, St. Petersburg, USA

Backgrounds

It is commonly recognized that the act of creating questions enhances learners' understanding of course materials and promotes deep learning. Student-generated questions that involved higher cognitive skills (compared to a simple recall) have been linked to self-directed learning and improved conceptual understanding.

Objectives

In this multi-year study, students designed multiple-choice pre-exam questions during a Microbiology course aimed at higher levels of learning, followed by their discussion. Students were instructed to construct questions on the higher domains of Bloom's taxonomy. We tested the hypothesis that this intervention improves student learning.

Methods

Learning gains were measured as student achievement on the exam following the intervention, and compared to student achievement on the traditional exam (prior to which a review session was focused on instructor-led recitation of the key concepts). Over the duration of the experiment 162 students chose to participate in this study from 2009 to 2015.

Conclusions

Following the intervention in all years, average grade on the post-intervention exam increased by 7.4% points. It is important to point out that not all students benefited equally from this activity. The lowest quintiles improved their scores on the second exam in a range of 6.8% to 9.9% percentage points, respectively. Students who were in the 3rd quintile based on the results of the first exam demonstrated the highest achievement improving their performance on an average by 12.3% percentage points. The students within the forth quintile improved their performance by 10.8% percentage points. Such gains were not observed in the semesters when the intervention was not implemented.

FEMS7-1744
Education / Professional Development / Policies

PROJECT-BASED LEARNING METHODOLOGY IN MEDICAL MICROBIOLOGY

E. Mateo[1], *E. Sevillano*[1]
[1]*Universidad del País Vasco UPV/EHU, Immunology- Microbiology and Parasitology, Leioa, Spain*

Backgrounds

«Research Project» is an annual subject of 6 ECTS included in the third year of the Degree of Medicine (School of Medicine and Nursery. UPV/EHU) in which our Department is involved. There is evidence that students with a research experience during their training acquire knowledge and skills that increase the probability of getting involved in research more successfully.

Objectives

The aim of this work was to implement a project-based learning methodology in this subject, with the students working on microbiology, and to analyse its impact along time.

Methods

The students were monitored in groups of 20 students, working in groups of 4-5 people. Given an initial scenario, they had to come up with a research idea related to medical microbiology and to carry out a research project, including writing a funding proposal, developing the experimental assays, and analyzing and presenting their results to a congress organized by the University. Continual assessment was performed by both students and teachers. A satisfaction survey was carried out to gather the students' opinion.

Conclusions

The overall results regarding to the classroom dynamics, learning results and motivation after a three year period of implementation were favourable. Marks were higher after the implementation. The students referred more implication in the subject and self- demanding, and a greater capacity to interpret the results following the scientific method. They stated a greater interest about research than they had before coursing this subject. In addition, all of them would choose the project based methodology versus the traditional one.

FEMS7-0913
Education / Professional Development / Policies

SWI@SPAIN: A VERSATILE PLATFORM FOR STUDENTS OF DIFFERENT AGES AT HIGH SCHOOL LEVEL TO AWARE ABOUT THE WORLDWIDE ANTIMICROBIAL RESISTANCE PROBLEM

F. Navarro-García[1], C.C. Casares-Salve[1], R. Clemente-Caballero[1], C. González-Pérez[1], M. Ortega[1], L. Papineschi[1], A. Pérez-Domingo[1], J.H. Quispe-Abregú[1], L. Torroba-Vicario[1], J.L. Veiga-González[1], I. Navarro-Muñoz[1], R.M. Jiménez de la Vega[2], J. Medina[3], J. Pla[1]
[1]Departamento de Microbiologia II, School of Pharmacy, Universidad Complutense de Madrid
[2]Colegio Arturo Soria, C/Duque de Tamames-4., Madrid, Spain
[3]IES Alpajés, C/ de las Moreras- 1, Aranjuez, Spain

Backgrounds

SWI@Spain is the Spanish implementation of the Small World Initiative program (SWI) firstly developed in the USA. This educational program aims to expand into the society the importance of preserving antibiotics in view of the increase of the antimicrobial resistance among bacterial isolates throughout the world. Choosing High School students will help broaden the target audience in an attempt to spread the message into the society through their families.

Objectives

To evaluate the influence of the SWI program on the perception of microorganisms, the responsible use of antibiotics and the burden of antimicrobial resistance at different levels of High School students.

Methods

Two different high school groups (12 years-old, 1º ESO, and 17 years-old, 2º Bachillerato) were selected to implement the SWI program. Sessions were developed in the laboratories of selected schools in Madrid with the help of Degree students. Soils from different parts of Spain were obtained and analyzed for the presence of antibiotic-producing microorganisms, as well as ampicillin-resistant strains. This was financed by a project in educative innovation awarded by University Complutense de Madrid (INNOVA-Docencia 2016-2017, Project 40).

Conclusions

We will present the influence of this program in the perception of two groups of High School students of different ages about the use and importance of antibiotics through the analysis of questionnaires run before and after the sessions. The importance of soil microbiology will be highlighted as a method to find new antibiotics, as well as a possible origin of antimicrobial resistance.

FEMS7-1171
Education / Professional Development / Policies

PARTICIPATORY WORKSHOPS: THERE IS NOT AGE TO DISCOVER MICROBIOLOGY

M. Orruño[1], *I. Arana*[2]
[1]*Faculty of Pharmacy - University of the Basque Country UPV/EHU, Immunology- Microbiology and Parasitology, Vitoria-Gasteiz, Spain*
[2]*Faculty of Science and Technology - University of the Basque Country UPV/EHU, Immunology- Microbiology and Parasitology, Leioa, Spain*

Backgrounds

Every year the University of the Basque Country (UPV/EHU) organizes the Science Week (ZientziaAstea). This annual event, aimed at audiences of all ages, tries to bring science to a non-specialist public by means of workshops, scientific shows, conferences and other activities. The microbiology has been present in this event from the first edition.

Objectives

To try to transmit microbiology in a more efficient and participatory way.

Methods

In recent years we have launched 2 workshops aimed at children and adults. The first workshop we implemented, *Microorganisms working*, tries to refute the bad image of microorganisms and to highlight their benefits and their ecological and industrial relevance. Among others, attendees can taste several foods and beverages obtained with microbial participation and discover that microorganisms are involved in the production processes of numerous common household product.

As the format was not the most suitable to keep the attention of the younger participants, we decided to launch a new workshop specifically aimed at children between 6 and 12 years, *Small microbiology for small scientists,* which tries to introduce children the microbial world through games and activities. They can use a microscope to discover the microbial diversity in natural samples, simulate the bacterial growth or become aware of their own *microbiological fingerprint*.

Conclusions

The workshops have been quite successful, with all the available places filled and a high level of satisfaction among attendees. In addition, the children's workshop has been exported to other fairs and adapted to schools.

FEMS7-0741
Education / Professional Development / Policies

ISSUES OF INTEREST AMONG AUTHORS PUBLISHING IN MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES JOURNALS: IS MULTIRRESISTANCE A TREND TOPIC IN SPAIN?

M.E. Pablo-hernando[1], *S. Pablo-hernando*[2]
[1]*Hospital Severo Ochoa, Microbiologia, LEGANES, Spain*
[2]*Fundación BIOEF, Unidad de Investigación en Atención Primaria- Bizkaia, Bilbao, Spain*

Backgrounds

WHO states that “antimicrobial multirresistance is an increasingly serious threat to global public health that requires action across all sectors involved”. In Spain, 50% of antibiotic prescriptions are unnecessary and the time practitioners invest in antimicrobials and continuous training influences their performance.

Nowadays, scientific journals become the main mechanism to diffuse knowledge. They structure the functioning of academic communities, prioritize the research agenda and provide the training contains.**Objectives**

Thus, this descriptive longitudinal study aims to identify “interests” among authors contributing to Medical Microbiology journals, as an indirect approach to know whether “Multirresistance” attracts attention. Two questions are formulated:

- What topics EIMC authors have chosen to publish?

EIMC (*Enfermedades Infecciosas y Microbiología Clínica*) is the Clinical Microbiology and Infectious Diseases Spanish Society main publication.

- Are these issues considered in analogous European Journals?

Methods

Thoughts, experiences or comments are reflected on editor’s or scientific letters and their titles synthesize author’s perspectives.
“Iramuteq” (content-analysis software) has been used to analyze two groups of titles: EIMC with 1.792 headings and “European group” with 1.353 titles extracted from analogous journals: *European Journal of Clinical Microbiology and Infectious Diseases, Journal of Medical Microbiology*, *Infection* and *Scandinavian Journal of Infectious Diseases*.
This methodology helped to identify topics, to classify them creating thematic clusters and to study their progress over time.

Conclusions

Results show how HIV disease, methodology and severe infections have fed opinion articles over time, and how multirresistance has been unnoticed in Spain. This fact could be linked to the poor prescription outcome.

FEMS7-1843
Education / Professional Development / Policies

TAKING CHARGE OF MICROBIOLOGY PRACTICAL LESSONS IN HIGH SCHOOL AS A TRAINING FOR MASTER STUDENTS

D. Prieto[1], E. Roman[1], T. Fernandez-Acero Bascones[1], J. Pla[1]
[1]Dept. Microbiología II- School of Pharmacy- Universidad Complutense de Madrid, Microbiología II, Madrid, Spain

Backgrounds

The Small World Initiative (SWI) is an educational program based in Microbiology experiments that has been recently implemented in Spain (SWI@Spain). In the SWI@Spain version the experiments are carried out by High School students. These practical lessons are usually organized by undergraduate students and a Professor or a Researcher.

Objectives

To analyze the didactic suitability of applying the Small World Iniciative program (SWI@Spain) to Master students (MS) in accordance to the Master Program.

Methods

Volunteer students from the Master of Microbiology and Parasitology (UCM) were included in the SWI program, adopting the service-learning strategy (SWI@Spain). During the experience, different aspects about the teaching-learning process were collected from the supervisor and the MS and analyzed. The information obtained was compared with the aims and skills proposed from the Master program. This was financed by a project in educative innovation awarded by University Complutense de Madrid (INNOVA-Docencia 2016-2017, Project 40).

Conclusions

The participation in the SWI program was considered as positive or very positive, in general and in the context of the Master degree, by all the MS. The learning achievements derived from the SWI experience gained by the MS correlate with most of the aims and skills defined in the Microbiology and Parasitology Master Program. Besides, the practical essence and the active learning result in an interesting complement for the MS formation.

FEMS7-2456
Education / Professional Development / Policies

LOOKING FOR NEW ANTIBIOTICS: THE SMALL WORLD INICIATIVE IN SPAIN.

C. Rodríguez[1], B. Conde[1], V. Fernández[1], P. Higueras[1], M. Rodrigo[2], D. Ruiz[1], J.A. Valdés[1]
[1]Universidad Complutense Madrid, Microbiology II, Madrid, Spain
[2]Universidad Complutense Madrid, Microbiology III, Madrid, Spain

Backgrounds

One of the main problems in the 21th Century is the lack of new therapeutic agents for fighting against multi-resistant bacteria. Looking for antibiotics and doing science in high school education will have a significant impact in society, especially among young people. The *Small World Iniciative* (SWI-USA), now introduced in Spain (SWI@Spain) for the first time, aims the students to become increasingly aware of problems with the over prescription and misuse of antibiotics, and the failure of treatments. Moreover, this experience can approach them to science and stimulate young scientific vocations. This is also a system of self-learning and an excellent way to open their minds to the future.

Objectives

The main goal is to concern the students about the clinical, social and economic impact of the treatment of bacterial infections. They are also introduced to the scientific method, microbiological techniques and the research of new soil bacterial strains.

Methods

We have applied the SWI strategy in the *IES Madrid Sur*. One microbiology faculty member (SWI Partner Instructor, SWIPI) and six undergraduate and graduate students (SWI Teaching Asistants, SWITAs) have been working with 36 high school students, during five sessions, two hours each. We have a special interest in divulgation of science, particularly through social networks (Facebook, Twitter, and a public blog) and official websites. Financed by INNOVA-Docencia 2016-2017, Project 40.

Conclusions

We have found new interesting bacterial strains producing antibiosis against the ESKAPE microorganisms used as testers. These strains have been identified by microbiological and molecular methods.

FEMS7-1832
Education / Professional Development / Policies

PROMOTING SOFT-SKILLS DEVELOPMENT IN MICROBIOLOGY PRACTICAL CLASSES

B. Sampaio-Maia[1], P. Vieira-Marques[2], J.S. Maia[3], V. Teixeira[4]
[1]University of Porto, Faculdade de Medicina Dentária- I3S-
Instituto de Investigação e Inovação em Saúde- INEB-Instituto de Engenharia Biomédica, Porto, Portugal
[2]University of Porto, CINTESIS- Faculdade de Medicina, Porto, Portugal
[3]Universidade Lusófona de Humanidades e Tecnologias, CeiED-
Centro de Estudos Interdisciplinares em Educação & Desenvolvimento, Lisboa, Portugal
[4]Universidade de São José, Faculdade de Psicologia e Educação, Macau, Macau

Backgrounds

It is currently recognized that universities have limited impact on students' 'soft' skills development. Taking in consideration the present employment competitiveness, the soft-skills seem to assume great importance at the time of employer's recruitment. It is important, therefore, that the University promotes among its students the development of a wide range of complementary skills to their specialization area.

Objectives

To compare the impact of two teaching / learning strategies aiming at developing Microbiology students' soft-skills: A) Laboratory protocols - traditional laboratory practice, where students follow pre-defined protocols and B) Elementary Research Project, where students are challenged to formulate a research question and subsequently design and perform a study to answer that question (including laboratory work).

Methods

Microbiology students from Dentistry Bachelor plus Master from University of Porto performed both Laboratory protocols and Research Project methodologies. Data was collected trough a self-perception questionnaire (n = 39) evaluating 40 soft-skills scored on a Likert scale of 5 levels. Statistical analysis was performed by Wilcoxon assuming $p<0.05$.

Conclusions

In students' perception, the Research Project contributed more than the Laboratory protocols to the development of soft-skills related to personal development (Initiative, Innovation and Creativity, Planning, Ability to question and Written Communication), collaborative work (Group Work and Influence / Persuasion skills), Information technologies (including Collection and Processing of Information) and Numeracy. These results suggest that challenging students with a Research Project in addition or in alternative to traditional Laboratory protocols foster a wider soft-skills development and encourage students' engagement.

FEMS7-0621
Education / Professional Development / Policies

#MICROMOOCSEM: A MICROBIOLOGY MASSIVE ONLINE OPEN COURSE VIA TWITTER (1ST EDITION)

I. López-Goñi[1], M. Sánchez[2]
[1]Universidad de Navarra, Microbiología y Parasitología, Pamplona, Spain
[2]Universidad Miguel Hernandez, Dpt. Vegetal Production and Microbiology, Elche - Alicante, Spain

Backgrounds

Social networks are increasingly used by the population on a daily basis. They are considered a powerful tool for Science communication. However, there are yet scarce examples on the use of Twitter in Science teaching and communication.

Objectives

Here we present and analyze the results of our pioneering experience imparting a full Basic Microbiology course via Twitter using the hashtag #microMOOCSEM (1st edition).

Methods

The course has consisted of 29 sessions, involving 1,225 tweets, 702 images, 265 hyperlinks to web pages and 136 videos related to Microbiology. Each lecture consisting of a series of 30-50 sentences (tweets) was programed to be of 40-45 minutes, at a one tweet per minute rate, along 10 weeks. Students were encouraged to follow the course through their mobile devices or computers, either live at 22:00 h (GMT+1) or later, following the hashtag.

Conclusions

Data analyses on the impact and acceptance of the course are encouraging, promoting a 330% enhancement in the followers of the Twitter account of the host institution, and reaching almost one third of overseas visits among the total amount of roughly 175,000. Certain classes became a Twitter *trending topic* in Spain.
Massive online open course via twitter in the format we adopted are highly dynamic, interactive and accessible to great audiences, providing a valuable tool for social learning and communicating Science, promoting the interest of students towards particular topics in the field, and complementing academic activities, especially in multidisciplinary areas like Microbiology.
A new proposal for a European edition will be presented.

FEMS7-1600
Education / Professional Development / Policies

"MICRO-MOVIE CLIPS", A STRATEGY TO IMPROVE CLASS ATTENTION

M. Sánchez[1]
[1]*Universidad Miguel Hernandez, Dpt. Vegetal Production and Microbiology, Elche - Alicante, Spain*

Backgrounds

One of the main challenges that any teacher faces when he starts giving his class time is to get the attention of the students. Some situations (e.g., first time in the morning or last class of the day) have in common that the student has difficulty paying attention at the beginning of the class, a period of time that the teacher uses to introduce the subject to be exposed that day setting the tone of the explanation later.

Objectives

Several strategies have been used to attract the attention of the students in those first moments (KA Feldman, 2013). Based on previous results (M Sánchez, 2011) I have used several movie clips from famous films to introduce the different lectures on the Industrial Microbiology course in order to increase the interest on the subject.

Methods

30 different "micro-movie clips" were used. Most of them were directly related to the topic to be dealt with on that day - e.g., a clip from "Extraordinary Measures" (T. Vaughan 2010) was used for introducing the lecture on Biotechnology Entrepreneurship. This teaching strategy has been used from the 2013 course to this day. The results were evaluated through a voluntary and anonymous survey conducted among the students.

Conclusions

Around three quarters of the students that completed the survey answered "It was very interesting and helped me understand the subject that was presented that day ". After the implementation of this strategy the percentage of exam failure never exceeded the 15%.

FEMS7-2535
Education / Professional Development / Policies

SMALL WORLD INITIATIVE AT THE DEUTSCHE SCHULE MADRID: CITIZEN EDUCATION TO FIGHT THE ANTIMICROBIAL RESISTANCE

C. Serna Bernaldo[1], I. Sánchez Méndez[1], C. Valenzuela Agüi[1], R. Martín[2], F. Müller[2], B. González-Zorn[1]
[1]Universidad Complutense de Madrid, Departamento de Sanidad Animal- Facultad de Veterinaria, Madrid, Spain
[2]Deutsche Schule Madrid, Biología, Madrid, Spain

Backgrounds

Antimicrobial resistance is an issue of international concern that will impact all of us. Discovering novel antibiotics and improving awareness and understanding of this problems are some of the strategies to avoid the spread of antibiotic resistance. The Small World Initiative ™ (SWI) started at Yale University in 2012 with the purpose of increasing public awareness among students (not only healthcare professionals), in addition to the possible discovery of new molecules acting as antibiotics from soil bacteria.

Objectives

We are students of the Veterinary School at University Complutense of Madrid (Spain). For this project, we have woked with the Deutsche Schule Madrid, focusing on pre-university students, 17-18 years old. Our main aim is to teach our knowledge about antimicrobial resistance to highschool students, encouraging them to start taking an active part in combating resistance and getting them involved in the scientific world. Besides, due to our Veterinary studies, we encourage a One Health approach which explains and fights antibiotic resistance in humans, animals and environment.

Methods

Soil samples were collected from various locations throughout the Madrid region. Bacteria from soil were isolated, and then cultivated with ESKAPE-like bacteria to evaluate if any of the isolated bacteria would produce an antibiotic substance. After that, potential candidates of antibiotic producer were identified with molecular techniques.

Conclusions

This poster will be used to describe the antibiotics producers found in this study.

FEMS7-1791
Education / Professional Development / Policies

TESTS ON LINE DURING THE CLASSROOM TEACHING TO INCREASE THE ATTENTION AND UNDERSTANDING OF THE SUBJECT

E. Sevillano[1], R. San Millán[1], E. Eraso[1]
[1]Universidad del País Vasco UPV/EHU, Immunology- Microbiology and Parasitology, Bilbao, Spain

Backgrounds

During the last years, new tools to increase the active participation of the students and the teacher/student interaction have been introduced. These methodologies have evolved allowing real time participation of the students answering questions by electronic devices that enables to get an immediate insight into students understanding.

Objectives

The aim of this work was to formulate multiple choice questions via Socrative Application after each lecture of the subject «Microbiology and Immunology» programmed in the first year of the Dentistry Degree in the University of the Basque Country (UPV/EHU).

Methods

After each lecture, the students were asked to log in the application with their mobile phones, tablets or computers, and to answer 4-5 multiple choice questions. The results were obtained in real time by the teacher and the wrong answers or possible doubts were cleared up straightaway. Students were presented with a satisfaction survey for feedback on the usefulness of the methodology.

Conclusions

The results obtained showed a global positive evaluation with 93.4% of students that would repeat the experience, and from 74 to 80% thinking that the methodology helped them to a better understanding of the subject, to pay more attention during the lectures and as a practice for the exam. Socrative was a very intuitive application and easy to use for the 95% of students. Most of them (80%) think that this learning experience had contributed more to the comprehension of the subject than traditional methodologies. Tests on line are useful in the university teaching activity for improving the learning process.

FEMS7-0922
Education / Professional Development / Policies

STANDARDISED METHODS IN PRACTICAL LAB WORK GENERATES IMPORTANT KNOWLEDGE FOR STUDENTS, SCIENTISTS AND THE FOOD SAFETY AGENCIES

C.S. Svanevik[1], *B.T. Lunestad*[1]
[1]*National Institute of Nutrition and Seafood Research, Contaminants and Biohazards, Bergen, Norway*

Backgrounds

The background for this work was to use standardised laboratory methods for both practical and theoretical teaching in seafood microbiology, where the achieved data also generates new knowledge for scientist and the government. As contaminated water is globally the main vehicle for microbial pathogens in most regions, we find that teaching future microbiologist and employees in the food industry on the importance of hygienically satisfactory water, microbiological analyses and how to ensure good water quality and safety, is highly relevant.

Objectives

This work present a complete experimental design for water analyses as a tool to teach students the methods and other key elements in microbiology, including food safety, environmental dissemination and survival of microorganisms, laboratory practices, water legislation and critical evaluation of results.

Methods

Analyses for the detection culturable bacteria (ISO 6222) and of fecal contamination in water (ISO 4788:1990, 4792:1990, 7899-2:2000) were used as an educational tool during a University course in seafood microbiology over a ten-year period (2006-2015). In addition to the lab results, the achieved knowledge among the students was examined by two retrospective questionnaires (about water analysis and lab course) conducted during June 2015. The questionnaires were circulated and made available for students back to 2006.

Conclusions

The questionnaires revealed that the laboratory course is highly appreciated, and that many students remembered important aspects of the water analysis, even after several years. The questionnaire results were consistent with our perception that some students find calculation of dilutions difficult to comprehend.

FEMS7-0976
Education / Professional Development / Policies

HANDS-ON STAINING TECHNIQUES TO LEARN ABOUT BACTERIA

A. Martins[1,2], C. Dias[1,2], M.J. Fonseca[2,3], F. Tavares[1,2]
[1]Faculdade de Ciências da Universidade do Porto, Departamento de Biologia, Porto, Portugal
[2]CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos/InBIO Laboratório Associado, Microbial Diversity and Evolution, Porto, Portugal
[3]Universidade do Porto, Museu de História Natural e da Ciência da Universidade do Porto MHNC-UP, Porto, Portugal

Backgrounds

Practical work-based approaches have long been regarded as privileged educational tools in biology education. These practices lead to opportunities for students to develop conceptual, procedural and attitudinal skills.

Objectives

1. Assess the impact of a practical approach based on staining procedures on the learning process concerning bacteria;
2. Clarify misconceptions about bacteria;
3. Acquaint students with microscopy and staining techniques;
4. Foster the development of critical observational skills.

Methods

The activity, trialed and tested with 15/16 year-old students, included the observation and identification of prokaryotic cell structures using the optical microscope, and microscope slides prepared by the students and from collections. Data was gathered through a pre-/post-test design mixed-method approach with the use of an out-group. Statistical analyses were carried out using IBMS' SPSS v.21.

Conclusions

The findings revealed the overcoming of frequent bacteria-related misconceptions. Experimental group students listed notions such as "show non defined nucleus" [16 (post-test)vs.11(pre-test);$\chi 2(1)=12.07;p<0.001$] or "with capsule which confers resistance" [6 vs. 0;$\chi 2(1)=5.14$; $p=0.02$]. Both groups agreed that microscopy is important to diagnose bacteria (Expgroup:M=4.27;Dp=1.20;Z=340.5;p<0.001,d=0.14; Cntgroup:M=4.48;Dp=0.70;Z=300; p<0.001, d=0.01) and perceived laboratory work as an important learning tool (Expgroup:M=4.90; Dp=0.31;Z=465;p<0.001;d=0.42; Cntgroup:M=4.93; Dp=0.27;Z=378;p<0.001;d=0.50), allowing them to understand issues addressed in their classes (Expgroup:M=4.79;SD=0.48;Z=-1.42;p=0,15;d=0.16; Cntgroup:M=4.67;SD=0.48;Z=378;p<0.001;d=0.16). Regarding microscopy techniques, students emphasized their impact, namely at a motivational level, stating that these allow to "study bacteria in more detail".
The laboratory activity implemented had a positive effect in the participants' conceptual learning (morphology and physiology of the bacterial cell) and allowed the development of their procedural capabilities, as well as of their critical thinking skills.

FEMS7-0494
Education / Professional Development / Policies

DEVELOPING COMMON CURRICULUM AND IMPROVING TEACHING APPROACHES OF MICROBIOLOGY EDUCATION

A. Netrusov[1], A. Trchounian[2]
[1]M.V. Lomonosov Moscow State University, Microbiology, Moscow, Russia
[2]Yerevan State University, Biochemistry- Microbiology & Biotechnology, Yerevan, Armenia

Backgrounds

Microbiology is one of the major subjects in Biology Education. New achievements of biology and formed basic and applied microbiology problems, on the one hand, the mobility of students and the labor market demands in microbiology specialists, on the other hand, require the development of a common (European or international) curriculum of academic microbiology [1].

Objectives

Microbiology in undergraduate education appears in two directions (areas) - General Microbiology and Applied (Medical) Microbiology.

Methods

By study and comparison of the microbiology programs for different universities, the following is proposed.

Conclusions

The new curriculum of General Microbiology may include three modules: (1) structure of the microbial cell (morphology), physiology, biochemistry and genetics of microorganisms; (2) biodiversity, ecology and systematics of microorganisms; (3) types, methods and directions of microbial biotechnology. A certain part of the program (to a quarter of volume) can include regional microbiology problems associated with microbial diversity, prevention of microbial diseases and environmental problems. It is important to highlight the objectives and output to ensure practical relevance. Special courses in undergraduate and graduate microbiology education in-depth subjects are in two areas and three modules.

To improve teaching approaches the problematic (interactive) lectures, practical classes and laboratory work, tests, and various forms of individual work should be implemented. The ratio of lectures and laboratory work should be offered approximately equal. Laboratory work must be carried out accordingly by general (international) protocols. Also important are consistent actions for the implementation to include assessment of the knowledge and learning feedback.

[1] Recommended curriculum guidelines for undergraduate microbiology education. ASM, 2012.

FEMS7-0609
Education / Professional Development / Policies

COST EFFECTIVENESS ANALYSIS REFLECTS THE IMPORTANCE OF INFECTION CONTROL IN BURN CARE

T. Shin-Yi[1], L. Chon-Fu[2], H. Cheng-Wei[1], L. I-Hong[1], H. Yan-Jiun[1], Y. Chia-Meng[3], K. Chien-Feng[4], S. Shou-Chuan[5]
[1]Mackay Memorial Hospital, Department of Laboratory Medicine, Taipei, Taiwan R.O.C.
[2]Macao Centro Hospitalar Conde de Sao Januario- Macao,
Centro Hospitalar Conde de Sao Januario, Macau, Macau
[3]Mackay Memorial Hospital, Department of Plastic Surgery, Taipei, Taiwan R.O.C.
[4]Mackay Memorial Hospital, Department of Infectious Disease, Taipei, Taiwan R.O.C.
[5]Mackay Memorial Hospital, Department of Medical Research, Taipei, Taiwan R.O.C.

Backgrounds

There are profound differences in the cost of burn care all over the world.

Objectives

We aim to investigate the affected factors and to delineate the strategy to improve the cost-effectiveness of burn management.

Methods

A retrospective analysis of 66 patients suffering from acute burns was conducted from 2013 to 2015. The average age was 26.7 years old and TBSA was 42.1% (±25.9%). T-test and Chi-squared tests were used to compare clinical characteristics between patients with and without bacteremia. A univariate regression and a multiple regression sequentially were used to examine the relationship between a variety of dependent factors, and individual and total costs.

Conclusions

The group of patients with bacteremia, incidence rate as 18.2%, had larger total burn surface area (TBSA), inhalation injury and full thickness significantly and the cost in that group significantly increased, especially medication expenditure. Through a multivariate regression analyses (Adjusted R^2=77.2%) to develop a normogram which can estimate the total cost of acute burn management, as follows. Estimated cost of acute burn care (10000 TWD)= -19.80 + (2.67 x percentage of TBSA) + (124.29 x status of inhalation injury) + (147.63 x status of bacteremia) + (130.32 x status of respiratory tract infection)
Our findings are compatible the previous studies indicating drug expenditures, the vast majority related to the use of antibiotics, on burns care were found to be high. Consequently, it is crucial to prevent nosocomial infection in order to promote our healthcare quality and reduce the in-hospital cost.

FEMS7-1045
Education / Professional Development / Policies

SERVICE-LEARNING IN THE SMALL WORLD INITIATIVE PROGRAM: AN OPORTUNITY FOR UNDERGRADUATES TO LEARN MICROBIOLOGY BY TEACHING YOUNGER STUDENTS

M.J. Valderrama[1], M.I. de-Silóniz[1], G. Barcia[1], L. Caballero-Nadal[1], P. Calvin[1], S. Fernández[1], N. García[1], M. Gómez[1], R. Gómez[1], N. Martínez[1], E. Palomino[1], M. Pérez[1], C. Rodrigo[1], R. Rojas[1], G. Velayos[1]
[1]UNIVERSIDAD COMPLUTENSE DE MADRID, Dpt. Microbiology, MADRID, Spain

Backgrounds

The University plays an important role in transmitting knowledge in benefit of the society. In the novel educational strategy Service-learning the university students learn while participating in real community needs. This methodology has been used in the implementation in Spain of the Small World Initiative (SWI), an educational program focused on crowdsourcing antibiotic discovery which also aims to aware about the antimicrobial resistance problem.

Objectives

Our objective was to carry out a service-learning experience by getting university students fully involved in the SWI project, in order to promote a more significant learning of Microbiology.

Methods

University students from Biology, and Biochemistry Degrees and the Master in Education participated in the Project. Following SWI protocols they were made responsible for organizing and carrying out visits to Secondary Schools, where they would teach young pupils: preparing laboratory materials, explaining the antimicrobial resistance problem, demonstrating and supervising microbiological methods, conducting the discussion of results with the school students and their teachers, Surveys were made to evaluate the effects on the teaching/learning process.

Conclusions

The Project had a high positive impact on motivation and awareness of antibiotic resistance, and the University students acquired a deeper and longer-lasting knowledge of practical microbiology. The Master students, soon secondary school teachers, considered this challenge a very valuable educational experience. The main keys to this success were: becoming involved in the learning process rather than being passive receptors, being responsible for specific tasks and teaching other students.

FEMS7-1352
Education / Professional Development / Policies

DYNAMIZATION OF BACTERIAL METABOLISM AND TAXONOMY STUDY FOR BIOTECHNOLOGY STUDENTS AT FRANCISCO DE VITORIA UNIVERSITY

O. Zafra[1], *E. Pérez Lago*[1], *C. Santos*[1]
[1]*Universidad Francisco de Vitoria, Experimental Sciences, Pozuelo de Alarcón Madrid, Spain*

Backgrounds

Microbiology is a key subject for Biotechnology students. In Francisco de Vitoria University (UFV), is taught during the second year of the degree and for lecturers is a great challenge to transmit the metabolic diversity and versatility of microorganisms as a source of tools for industry, medicine and the development of welfare in society.

Objectives

During this academic year we are trying to evaluate the perception of the students about the subject, applying some didactic tools to raise it and quantify the improvement. As bacterial metabolism and taxonomy are the items usually thought as more difficult and boring, we developed a dynamic method to improve the teaching.

Methods

First of all, we designed an inquiry to quantify the perception of the student about the importance of the bacterial metabolism and taxonomy items. This inquiry was done before and after the application of the new didactic tools. In this case, we used an advanced organizer for the student to deduce the types of metabolic reactions based on Barbosa *et al*, 2005 and asked them to classify some taxonomic groups on it. This would help the student to relate the knowledge and fix it in a dynamic way.

Conclusions

As a general conclusion is worth to highlight that taxonomy clearly improved its rate in dynamism. In the poster presentation we will explain the method and the whole statistics.

FEMS7-0591
Education / Professional Development / Policies

ACCESS AND USE OF MICROBIAL RESOURCES: THE CONVENTION ON BIOLOGICAL DIVERSITY AND THE NAGOYA PROTOCOL

A. Zuzuarregui[1], R. Aznar[1]
[1]Colección Española de Cultivos Tipo - Universidad de Valéncia, Microbiology and Ecology, Burjasot Valencia, Spain

Backgrounds

The Convention on Biological Diversity (CBD) entered into force on December 1993 and has three main objectives:

- the conservation of biological diversity,

- the sustainable use of its components,

- and the fair and equitable sharing of benefits arising from the use of genetic resources.

The Nagoya Protocol (NP) is a supplementary agreement of the CBD and entered into force on October 2014. It provides instructions to implement the third objective of the CBD, e.g. the access to the genetic resources and the fair and equitable sharing of benefits arising from their utilization (ABS).

According to the CBD, the States have the sovereign right to exploit their own resources and may regulate access to such resources (procedure to apply for the Prior Informed Consent, PIC) and the conditions on which this access and the subsequent use are granted (Mutually Agreed Terms, MAT).**Objectives**

To raise awareness about the international regulations related with ABS and show a simplified flow diagram covering the main aspects to reach compliance.

Methods

Analysis of the regulations and internet resources:

- CBD https://www.cbd.int/
- PN https://www.cbd.int/abs/
- ABS Clearing House https://absch.cbd.int/
- European Commission's ABS information http://ec.europa.eu/environment/nature/biodiversity/international/abs/index_en.htm

Conclusions

Researchers collecting strains from countries Party to the CBD must observe their national regulations regarding access to genetic resources. If the country has put in place ABS measures, the researcher(s) must apply for the PIC and the MAT. The information about national legislative measures and national focal points for ABS issues is available at the ABS Clearing-House (ABSCH).

FEMS7-2234
Environmental Microbiology/Microbial Ecology /Microbial Communities

ANALYSIS OF RESISTANCE TO ERTAPENEM OF BACTERIA ISOLATED FROM THE TINTO RIVER ESTUARY (HUELVA, SPAIN) AND THE WATER FALL CHORRERA DE DESPENALAGUA (GUADALAJARA, SPAIN)

J.P. Abad[1], B. Eduardo-Correia[2], H. Morales-Filloy[3]
[1]Universidad Autónoma de Madrid, Departamento de Biología Molecular, Cantoblanco, Spain
[2]UKM Münster, Clinic of anaesthesiology- Intensive care and pain treatment, Münster, Germany
[3]University of Heidelberg, Institute of Pharmacy and Molecular Biotechnology IPMB, Heidelberg, Germany

Backgrounds

Resistance to antibiotics is an ever-growing threat that may be related to the abuse in the use of these substances and their arrival to the environment. Among the resistances to antibiotics are included those to antibiotics used as last resource as i.e. the carbapenems.

Objectives

The objective of our study was determining the prevalence of bacteria resistant to ertapenem among isolates resistant to ampicillin obtained from two aquatic biological systems, a contaminated and cuasi marine at SW Spain, and another cuasi-pristine and of fresh water at central Spain.

Methods

The growth of a collection of about 1000 isolates from both systems was tested at several concentrations of ertapenem (0.063-8.0 µg/mL) using solid nutritive or marine media. MIC values for each of the environmental isolates were determined based on the results obtained. Phylogenetic adscription was performed by partial sequencing of 16S rDNA.

Conclusions

Considerable percentages of the isolates were resistant to 2 µg/mL of ertapenem, 79.7% of the isolates from the Tinto river estuary and 43.5% of those from the Chorrera de Despeñalagua. These values increased when considering only bacteria isolated on marine medium from each system. The antibiotic MIC values were also higher for the isolates from the salty system than for those from the fresh water one. An effect of the medium composition was observed on the resistance to this antibiotic, which seems to be also related to the specific phylogenetic adscription of the isolates. The results will be reported and discussed.

FEMS7-0432
Environmental Microbiology/Microbial Ecology /Microbial Communities

METAGENOMIC AND FUNCTIONAL ANALYSES OF AIRBORNE MICROORGANISMS IN FINE PARTICULATE MATTER (PM2.5) DURING PM EVENT AND NON-EVENT DAYS

A. Abd Aziz[1], B. Park[2], H. Park[2], K. Lee[1], K. Park[1], I.G. Choi[2], I.S. Chang[1]
[1]Gwangju Institute of Science and Technology GIST,
School of Earth Sciences and Environmental Engineering SESE, Gwangju, Republic of Korea
[2]Korea University, College of Life sciences and Biotechnology, Seoul, Republic of Korea

Backgrounds

With a great concern on the risk of airborne particulate matter (PM) towards human health, especially the $PM_{2.5}$ (particulate matter of aerodynamic diameter < 2.5 µm), this study focuses on the biological components and changes in the air which may also impose threat towards human health.

Objectives

Whole metagenomics shotgun sequencing (WGS) was conducted to see whether the microbial community and metabolic functions of PM event day (designated daily $PM_{2.5}$ concentration > 50 µg m^{-3}) are changed in the sample compared to PM non-event day.

Methods

MG-RAST server was used to analyze the annotated sequences, and the results showed that *Bacteria* was dominant domain with 95% and 45%, respectively in both non-event and event day samples. Followed by *Eukaryota*, with 3.7% and 52%, respectively. Additionally, there was an increase to 1.9% of *Firmicutes* group in the PM event day sample. Under this phylum, there are opportunistic pathogenic species such as *Staphylococcus aureus* and *S. haemolyticus* were significantly detected. Furthermore, by comparing the annotated proteins within KEGG Orthologs functional category, functional genes related to metabolism was predominant for both samples at 55%. Also, the functional genes related to human diseases such as Prion diseases had newly detected in the PM event day sample.

Conclusions

The results of this study suggest the public should be more cautious attending outdoor activities during PM event days, because on such days more pathogens and allergens were detected as well as an increase in functional genes related to human diseases.

FEMS7-2128
Environmental Microbiology/Microbial Ecology /Microbial Communities

ANTIMICROBIAL EFFECT AND BIOCOMPATIBILITY STUDY OF POLY(EPSILON-CAPROLACTONE) SUPERFICIALLY MODIFIED BY OXYGEN PLASMA AND BIOCIDE STRUCTURES

C. Abrusci[1], A. Morro[1], T. Corrales[2], J.L. Pablos[2], I. Marin[1], F. Catalina[2]
[1]Universidad Autónoma de Madrid, Biología Molecular, Madrid, Spain
[2]Consejo Superior de Investigación Científica, Instituto de Ciencia y Tecnología de Polímeros, Madrid, Spain

Backgrounds

The development of anti-infective biomaterials is regarded as the main strategy to prevent the bacterial colonisation and biofilm formation. In this study, poly(ε-caprolactone) (PCL) is superficially modified with antimicrobial active structures (tetrabutylammonium, ampicillin, silver cations and cetylpyridinium) which were prepared via a two-step process. Firstly, PCL was exposed to an oxygen plasma for surface activation, and then immersed into aqueous solutions of biocides for their adsorption.The antibacterial effect of oxygen plasma treatment and the biocides loaded onto the surface were evaluated by measuring the adhesion and growth of two clinically significant bacteria *Bacillus cereus* and *Pseudomonas aeruginosa*. In order to assess the degree of biocompatibility, the adhesion and proliferation of murine L929 fibroblasts were studied by performing *in vitro* and *in vivo* assays. Also, cell viability and cell death assay were carried out.

Objectives

To design anti-infective and biocompatible PCL-based materials.

Methods

The modified PCL materials were characterized by different techniques (ATR-FTIR, Chemiluminescence, SEM, AFM). In this study, the biophysical responses of bacteria and murine cell fibroblasts towards the prepared material films as model device surfaces were elucidated.

Conclusions

The obtained results demonstrated that, except for the materials loaded with silver cations and cetylpyridinium, the PCL materials prepared in this work are adequate as anti-infective biomaterials.

FUNDING-MAT2012-31709- MINECO-FEDER-EU

FEMS7-2184
Environmental Microbiology/Microbial Ecology /Microbial Communities

STUDY OF INHERENT ANTIMICROBIAL ACTIVITY AND IN VITRO BIOCOMPATIBILITY OF COPOLYMERS FILMS CONTAINING SULFADIAZINE ACRYLIC MOIETIES

C. Abrusci[1], A. Morro[1], J.L. Pablos[2], I. Marin[1], F.C. García[3], J.M. García[3]
[1]Universidad Autónoma de Madrid, Biología Molecular, Madrid, Spain
[2]Consejo Superior de Investigaciones Científicas, Insitituto de Ciencia y Técnología de Polímeros, Madrid, Spain
[3]Universidad de Burgos, Departamento de Química, Brugos, Spain

Backgrounds

Microbial colonisation of synthetic material, is a great concern in many fields, e.g., in implant surgery and medical devices; therefore biocompatible hydrophilic organic materials with inherent antimicrobial and anti-biofilm properties are of current research interest.In this work, we describe the preparation of antimicrobial and biocompatible polymeric film based on *N*-vinyl-2-pyrrolidinone (VP) and 2-hydroxyethyl acrylate (HEA), using ethyleneglycol dimethacrylate (EGDMA), and synthetic acrylic monomer containing sulfadiazine chemically anchored.

Objectives

To design a PVP-based polymer with antibacterial properties.

Methods

The synthetized PVP-based films were characterized by different techniques (^{1}H and ^{13}C NMR, ATR-FTIR, SEM, TGA). In this study, the biophysical responses of bacteria and L929 cells towards the prepared materials as model device surfaces were evaluated.

Conclusions

The polymers obtained showed excellent antibacterial activity against *E.coli*, with inhibition values higher than those of silver and allowed to control the biofilm formation that can cause medical device contamination by *E.coli*. Cell adhesion and cytotoxicity studies assessed the biocompatibility of the polymer, being a good candidate for medical applications as a biomaterial.

FUNDING- MAT2014-54137-R ; MAT2012-31709and BU061U16 - MINECO-FEDER-EU

FEMS7-1887
Environmental Microbiology/Microbial Ecology /Microbial Communities

MOLECULAR MICROBIOLOGY APPROACH TO ASSESS THE BIODEGRADATION POTENTIAL OF AN ENVIRONMENTAL INOCULA FOR ORGANIC POLLUTANT

K. Acharya[1], J. Dolfing[1], P. Meynet[1], W. Mrozik[1], D. Werner[1], R. Davenport[1]
[1]Newcastle University, School of Civil Engineering and Geosciences, Newcastle Upon Tyne, United Kingdom

Backgrounds

Activity of potential degraders is one of the major factor that determines the biodegradation potential on an environmental inocula for any organic pollutant. Little attention has been given to the investigation of the activity of potential degraders in mixed microbial communities in the degradation of organic pollutants.

Objectives

Our work focuses on the development of a robust tool to estimate and understand the biodegradation potential of activated sludge for phenol and 2,4-dichlorophenol, as model organic pollutants.

Methods

Batch laboratory assays were conducted in 500 mL glass bottles containing activated sludge from different Waste Water Treatment Plants (WWTP) and spiked with known concentration of model chemicals. Chemical concentration, CO_2 development in the headspace, DOC and total bacteria count were monitored and quantified over time. The key catabolic genes involved in the model chemical biodegradation pathway were identified from literature, specific primers were developed and used for the quantification in the batch reactors, using molecular techniques (e.g., qPCR and RT-qPCR).

Conclusions

Phenol was completely mineralized in the activated sludge from all WWTPs and followed 1st degradation kinetic, whereas 2,4-dichlorophenol was only degraded in one WWTP activated sludge. There was significant increase in the catabolic genes copies number over the duration of experiment ($p < 0.05$) and expressed significantly. Dichlorocatechol-1,2-dioxygenase (catabolic gene for 2,4-dichlorophenol biodegradation) gene were not detected in activated sludge not supporting 2,4-dichlorophenol degradation. Our results suggest that key catabolic genes involved in the pollutant biodegradation can be a biological marker to access the biodegradation potential of an environmental inocula against that pollutant.

FEMS7-2242
Environmental Microbiology/Microbial Ecology /Microbial Communities

EMERGING INSIGHTS INTO THE TYPE IV CRISPR/CAS SYSTEMS FROM GENOMIC ANALYSES THE ACIDITHIOBACILLUS SPECIES COMPLEX

L.G. Acuña[1], P.C. Covarrubias[1], A. Moya-Beltrán[1], O. Salgado[1], H. Nuñez[1], R. Quatrini[1]
[1]Fundacion Ciencia & Vida, Microbial Ecophysiology Laboratory, SAntiago, Chile

Backgrounds

Many bacteria and most archaea have genetically encoded Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) regions and CRISPR-associated proteins (Cas) that confer immunity to viruses and other mobile genetic elements. According to recent classifications the extreme acidophiles, *Acidithiobacillus caldus*^{T} and *A. ferrooxidans*^{T}, harbor Type U CRISPR/Cas loci on integrative conjugative elements present in their genomes. Both systems are only partially conserved between each other and other sequences deposited in public databases.

Objectives

Very little insight has been gained to date on the general occurrence, evolution and function of these systems. To advance in this direction we profiled the CRISPR/Cas systems in an extensive set of strains spaning the *Acidithiobacillus* species complex.

Methods

For that purpose we collected available genomic sequences, and screened experimentally additional strains of the genus. The CRISPR/Cas systems were characterized bioinformatically using comparative genomics stategies.

Conclusions

Nearly 50% of the strains analyzed (63 strains) harbored CRISPR/Cas systems in their genomes. Class IV (type U) system was widespread within the group (22% of strains spanning 5 linages), followed by Class 1 subtypes I-C (10%), I-E (10%) and I-F (6%), which were restricted to fewer taxa. Variant forms of the type U system were identified in the acidithiobacilli, providing hints on the inherent diversity and nature of this unusual class. Herein, we explore relevant aspects of the genes, repeats and spacers that conform these enigmatic CRISPR/Cas systems in the context of the emerging phylogenetic structure of the taxon.

Acknowledgements: Fondecyt 1140048, Basal CCTE PFB16 and UNAB and Conicyt Scholarships.

FEMS7-0822
Environmental Microbiology/Microbial Ecology /Microbial Communities

A GREENHOUSE ASSESSMENT OF COAL MINE SOIL STOCKPILES: ARBUSCULAR MYCORRHIZAL FUNGAL SPORE DENSITY AND COLONIZATION IN MAIZE (ZEA MAYS) TRAP CULTURES

O. Ezeokoli[1], R. Adeleke[2], C. Nwangburuka[3], A. Roopnarain[2], M. Maboeta[1], C. Bezuidenhout[1], D. Paterson[2]
[1]North-West University, Environmental Sciences and Management, Potchefstroom, South Africa
[2]Agricultural Research Council-Institute for Soil- Climate and Water, Soil Health, Pretoria, South Africa
[3]Babcock University, Department of Agriculture- School of Science and Technology, Ogun State, Nigeria

Backgrounds

Arbuscular mycorrhizal fungal (AMF) symbiotic colonization of roots is essential for nutrient uptake and growth of most vascular plants. Successful establishment of AMF mycorrhization in plant roots is dependent on the soil condition and soil management practice.

Objectives

This study investigated AMF spore density and colonisation in maize (*Zea mays*) trap cultures grown in stockpile soils obtained from three South African opencast coal mines

Methods

Stockpiles were randomly sampled at depths of ≤20 cm (topsoil) and >20 cm (subsoil), and used for mycorrhizal trap culture experiments in the greenhouse. Following a 12-week post germination period, AMF spore density in pot soils was enumerated, while plant roots were assessed for AMF colonisation and diversity by using classical staining and targeted amplification and sequencing of the nuclear rRNA gene of the *Glomeromycota* phylum.

Conclusions

AMF spore density was significantly different ($P<0.05$) between soil samples and highest in topsoil from unmined soils (control soil). The AMF spores were morphologically identified as belonging to the genera *Acaulospora*, *Gigaspora* and *Scutellospora*. DNA-based detection revealed that AMF colonisation was more associated with topsoils than with subsoils. Furthermore, species of the genus *Paraglomus* were the only colonisers of the maize roots in all soils, suggesting a very low diversity of viable AMF spores, poor support for the establishment of root-AMF symbioses or the presence of a single maize (host)-specific AMF symbiont in these soils. Overall, the results suggest that stockpiling and stockpile depths may have an effect on spore density and mycorrhization

FEMS7-1048
Environmental Microbiology/Microbial Ecology /Microbial Communities

AN ASSESSMENT OF BACTERIOPHAGES SPECIFIC TO E. FAECALIS AND E. FAECIUM AS POTENTIAL MARKERS TO DETECT FAECAL CONTAMINATION IN WATER

M.R. Adem[1], A. Kumar[1], C.N. Ateba[1]
[1]North-West University Mafikeng Campus, Biological Sciences, Mahikeng, South Africa

Backgrounds

Enterococcus species reside in the gastrointestinal tract of humans and animals have been used as indicators of faecal contamination in water. Due to the fact that bacteria counts are usually not reliable and reproducible bacteriophages are now used as potential tools for assessing bacterial contamination.

Objectives

This study was designed to evaluate the potential of *E. faecalis and E. faecium* and their specific phages as indicators water contamination.

Methods

Methods: A total of 162 water samples were collected and analysed and 119 presumptive enterococci were subjected to Gram staining, growth in 6.5% NaCl, oxidase and catalase as well as *E. faecalis* and *E. faecium* species specific PCR analysis (*ddl* gene PCR). *E. faecalis* and *E. faecium* specific bacteriophages were also isolated using standard methods. Phage morphologies were determined using electron microscopy. The antimicrobial resistance profiles of enterococci were determin**ed.** Results: A large proportion 50 (42%) of the isolates were confirmed as *E. faecium* while 16 (13%) were *E. faecalis*. Large proportions (62.1% to 100%) of these isolates were resistant to Erythromycin, Ampicillin, Gentamycin, Tetracycline and Chloramphenicol. However, only 27 (41%) and 12 (18.2%) of the isolates were resistant to Vancomycin and Amocyxillin respectively. Cluster analysis of 66 isolates produced two major clusters (Cluster 1 and Cluster 2). Resistance data indicated that isolates shared similar profiles. *E. faecalis* and *E. faecium* specific bacteriophages were detected and TEM data indicated that morphologies were similar to those of the family *Siphoviridae*.

Conclusions

These bacteriophages can be reliable indicators of faecal pollution in water.

FEMS7-2904
Environmental Microbiology/Microbial Ecology /Microbial Communities

IMPACT OF INOCULATION / EARTHWORM INTERACTION ON GROWTH AND PHYTOREMEDIATION CAPACITY OF ACACIA MANGIUM CULTIVATED ON POLLUTED SOIL LANDFILL

A.J. Bongoua-devisme[1], C. Balland Bolou Bi[2], F. N'doye[3], S.A. Kouakou[4], D. Diouf[5], B.E. Bolou Bi[4], B. Adiaffi[6], A. Yao-kouame[7], E.M.V. Djagoua[8]
[1]Université Félix Houphouët-Boigny- UFR STRM- 22 BP 582 Abidjan 22- Côte d'Ivoire., Département des Sciences du Sol, Cocody- Abidjan, Ivory Coast
[2]Université Paris Est Créteil- UPEC- UMR BIOEMCO – 61- Avenue du Général de Gaulle- 94010 Crét eil Cedex- France., Créteil, France
[3]Université Cheikh Anta Diop de Dakar- Institut Sénégalais de Recherches Agricoles ISRA- BP 1386- CP 18524 Dakar- Sénégal, Laboratoire Commun de Microbiologie, Dakar, Senegal
[4]Université Félix Houphouët-Boigny- UFR STRM- 22 BP 582 Abidjan 22- Côte d'Ivoire., département des sciences du sol, Cocody- Abidjan, Ivory Coast
[5]Université Cheikh Anta Diop de Dakar- Institut Sénégalais de Recherches Agricoles ISRA-, Laboratoire Commun de Microbiologie LCM, Dakar, Senegal
[6]Université Félix Houphouët-Boigny- UFR STRM- 22 BP 582 Abidjan 22- Côte d'Ivoire, Département Hydrogéologie, Cocody Abidjan, Ivory Coast
[7]Université Félix Houphouët-Boigny- UFR STRM- 22 BP 582 Abidjan 22- Côte d'Ivoire., Département des Sciences du Sol, cocody Abidjan, Ivory Coast
[8]Université Félix Houphouët-
Boigny- UFR STRM- Département des Sciences du Sol- 22 BP 582 Abidjan 22- Côte d'Ivoire., Géologie marine, Cocody- Abidjan, Ivory Coast

Backgrounds

In Côte Ivoire, most of the landfills are open air and contain different categories of wastes (municipal wastes, hazardous wastes, non-hazardous wastes). This practice can result in air, soil and groundwater pollution, with a potential risk to human and environment. Because of this problem, there is a great interest to rehabilitate of these polluted sites.

Objectives

The goal of this work is to test two catalyzers: earthworm (P. corethrurus) and symbiotic microorganism (*Bradyrhizobium)* on the processes of phytoremediation.

Methods

To obtain this objective, an experimental system are realized coupling P. corethrurus/ *Bradyrhizobium* with the plant *Acacia mangium* to study if this this combination can decontaminate the soils contaminated with ETM (Cr and Ni).

Conclusions

The results obtained after (03) months of culture in jars controlled condition, show that the pollution affects the vegetative development of *A.mangium*, but in presence of earthworm and *Bradyrhizobium* a better vegetative development had been noticed. Furthermore, a high content of metals (Cr and Ni) are been determined in plant (roots, sterm and leaves) in presence of the two catalyzer. However, our results reveal a translocation of Cr in leaves and stems and an accumulation of Ni in the roots. These results show the capacity of phytoextraction and phytostabilisation of *Acacia mangium*.

FEMS7-2449
Environmental Microbiology/Microbial Ecology /Microbial Communities

GENOMIC FEATURES OF BACTERIAL STRAINS UNIQUELY COMPATIBLE WITH PEA (PISUM SATIVUM L.) LINES WITH RESTRICTIVE SYMBIOTIC PHENOTYPE

A. Afonin[1], *A. Sulima*[1], *V. Zhukov*[1], *I. Tikhonovich*[2]
[1]*All-Russia research institute for agricultural microbiology, Laboratory for genetics of plant-microbe interactions, St-Petersburg, Russia*
[2]*All-Russia research institute for agricultural microbiology, Department of biotechnology, St-Petersburg, Russia*

Backgrounds

Garden pea (*Pisum sativum L.*) is a plant capable of forming nitrogen fixing nodules through beneficial symbiosis with *Rhizobium leguminosarum*. Various pea cultivars differ in the range of compatible micro symbionts - evidence of the everlasting arms-race between the plant and bacteria. Some pea lines of middle-eastern origin are highly selective in the choice of symbiotic partner, in some instances due to a naturally occurring allele of the *Sym6* gene. Strangely enough, similar heightened specificity can also be exhibited by mutants in *Sym37* and *Sym26* symbiotic genes. The genetic traits, making bacteria capable of nodulating these restrictive lines previously described as non-nodulating, are as of yet unknown.

Objectives

The aim of this work was to characterize and compare genomes of strains A1, RCAM1026, 3841, R.A.17 and R.A.11 to identify the genetic bases of strain-to-line specificity.

Methods

Strains were tested for their ability to form nodules with pea lines carrying restrictive alleles of genes *Sym37* (RisNod4 and K24), S*ym26* (P61) and *Sym6* (line 2150). Genomes of the strains were sequenced, assembled and analysed. Genome comparison showed a number of unique traits, possibly responsible for unique symbiotic properties of the strains.

Conclusions

Bacterial genome determines the compatibility between pea lines and bacterial strains. High number of secretion systems in the strain 3841 may be the cause of its' incompatibility with the P61 line. Strains A1 and RCAM1026, although similar in genetic composition possess a number of unique genetic characteristics, defining their host range.
This work was supported by Russian Science Foundation [grant 14-24-00135].

FEMS7-2575
Environmental Microbiology/Microbial Ecology /Microbial Communities

RESPONSE OF BACTERIAL COMMUNITIES FROM OCEANIC ENVIRONMENTS TO ELEVATED PCO2 AND LOW PH: A MICROCOSM APPROACH

P. Aguayo[1,2], D.L.I. Rodrigo[3], H. Carlos[4], U. Osvaldo[4], V. Cristian[4]
[1]Millennium Institute of Oceanography, Oceanography, concepcion, Chile
[2]Centro EULA Chile, Sistemas Acuáticos, Concepcion, Chile
[3]Millennium Institute of Oceanography,
Molecular Genetic and Microbiology - Pontificia Universidad Católica de Chile, Santiago, Chile
[4]Millennium Institute of Oceanography, Oceanography, Concepción, Chile

Backgrounds

Ocean acidification (OA) is one of the global issues caused by rising atmospheric CO2. The rising pCO2 and resulting pH decrease has altered ocean carbonate chemistry. Microbes are key components of marine environments involved in nutrient cycles, carbon flow in marine ecosystems. However, these marine microbes and the microbial processes are sensitive to ocean pH shift. Considering that there is little information on how communities of marine bacteria and archaea respond to chemical changes in the ocean and how these changes interfere with biogeochemical cycles

Objectives

It was postulated that CO_2 changes and the consequent decrease in ocean pH values modify the diversity of marine microorganisms .The response of the communities to the was evaluated by the use of microcosms the pH levels investigated were in situ seawater pH (7,99–8.05), pH 7.82 and pH 7.67, representing the present-day situation and two acidification scenarios projected for the year 2100.

Methods

The results obtained from the molecular analyses will be contrasted with abundance and composition of photosynthetic eukaryotes, heterotrophic bacteria from water samples and low and high pCO_2 treatment

Conclusions

The results indicate that there is a marked difference between controls and treatments, as well as it is possible to observe that bacterial communities respond rapidly to changes in ocean chemistry than eukaryotic photosynthetic communities. results generated will assess vulnerability and resilience of microorganisms against changes resulting from acidification, and the consequences that could have for the rest of the biological community that depends on this link key ocean food webs and ecosystem.

FEMS7-1432
Environmental Microbiology/Microbial Ecology /Microbial Communities

BACTERIAL COMMUNITY STRUCTURE AND FUNCTION IN THE WATER OF THE SODA SALINE CRATER LAKE FROM ISABEL ISLAND, MEXICO

J.F. Aguirre Garrido[1], H.C. Ramírez Saad[2], F. Martinez-Abarca Pastor[3], N. Toro García[3]
[1]Universidad Autonoma Metropolitana, Ciencias Ambientales, Lerma de Villada, Mexico
[2]Universidad Autonoma Metropolitana, Sistemas biológicos, Ciudad de México, Mexico
[3]Estación Experimental del Zaidín, Consejo Superior de investigaciones científicas, Granada, Spain

Backgrounds

Isabel Lake is a moderate saline soda crater lake located in Isabel Island in the eastern tropical Pacific coast of Mexico. Lake is mainly formed by rainfall and is strongly affected by evaporation and high input of nutrients derived from excretions of a large bird community inhabiting the island. The knowledge of the prokaryotic biota inhabiting the upper layers of this meromictic lake can give clues for the maintenance of this ecosystem.

Objectives

The aim of this work was to assess the diversity, composition and function of bacterial community in water of the Isabel Island crater lake in Mexico.

Methods

The study area is located in Isabel Island, a small volcanic island (2 km2) located 30 km off the Pacific coast of Mexico (21° 52′ N and 105° 54′ W). We sequenced three samples of water obtained around a dry season using a random whole-genome shotgun (WGS) approach and 16S rRNA amplicon masive sequencing using Illumina platform. Bioinformatic analyses were performed using MOTHUR version 1.33, STAMP, MG-RAST and MEGAN version 5.

Conclusions

The bacterial community is dominated by halophilic and halotolerant microorganisms. The lake water is dominated by γ- Proteobacteria belonging to four main families where Halomonadaceae presents the highest abundance. Aerobic, phototrophic, and halotolerant prokaryotes such as *Synechococcus, Rhodopirellula, Halomonas, Alcanivorax* and *Marinobacter* genera are commonly found. We identified several functions related with molybdate and zinc system permease proteins, antibiotic transport systems proteins.

FEMS7-0114
Environmental Microbiology/Microbial Ecology /Microbial Communities

WET WIPES WHICH IS SOLD IN MARKETS EVALUATION FOR THE MICROBIOLOGICAL RESPECT

B. Sapmaz[1], Ö. Akgül[2], P. Yüksel Mayda[3], R. Çalışkan[3], M. Samastı[3]
[1]Nişantaşı University, Vocational School, Istanbul, Turkey
[2]Üsküdar University, Vocational School of Health Services, Istanbul, Turkey
[3]Istanbul University Cerrahpaşa Medical Faculty, Medical Microbiology, Istanbul, Turkey

Backgrounds

Hands in continuous and close contact with the environment, they carry to a rich microbial flora. Neglected hand hygiene is caused the remaining of contamination of hands so inadequate implementation. The general purpose of hand hygiene is; temporary flora to be completely removed, reduction the number of permanent flora and thus to prevent transfer of bacteria via the hands.

Objectives

The aims of the present study was evaluate and compared the efficacy of wet wipes, soap with water, 70% alcohol and gel with alcohol against resident and transient flora.

Methods

We have investigated as compared different brands which do not contain the antiseptic wet wipes and including antiseptic wet wipes effects of resident and transient flora against of the washing with soap and water, 70% alcohol and gel with alcohol.

Conclusions

In our study, all forms of alcohol (liquid, gel and alcohol-impregnated wipes) by washing with soap and water was found to be much more effective. However, on the resident flora when compared to alcohol (62.5% and 65%) gel preparations, liquid alcohol (70%) and alcohol (70%) wipes form was less effective. Also, washing with soap and water eliminates the transient flora can be exactly that after the cleaning with a wet wipes in hand in excess of contaminant bacteria was found to remain. In the lights of our study, even the ensuring the hand hygiene have in many ways, alone wet wipes are not enough, water-washing facilities with soap and non-cases, preparations with antiseptic effect could be an alternative, but non antiseptic alone impurities can not be eliminated should not be forgotten either.

FEMS7-3011
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE COMPLEX AND ALARMING RESISTANCE STATUS ON CLOSTRIDIUM DIFFICILE IN TURKEY

Ö. Akgül[1], B. Sapmaz[2], F. Çatal[3], P. Yüksel[3], R. Çalışkan[3], H. Kırkoyun Uysal[4], Ö.F. Karasakal[1], G. Aygün[3]
[1]Üsküdar University, Vocational School of Health Services, İstanbul, Turkey
[2]Nisantasi University, Vocational School, Istanbul, Turkey
[3]Istanbul University Cerrahpasa Medical Faculty, Medical Microbiology, İstanbul, Turkey
[4]Istanbul University Istanbul Medical Faculty, Medical Microbiology, İstanbul, Turkey

Backgrounds

Clostridium difficile infection is commonly recognized as one of the most frequent nosocomial infections and has led to high mortality, heavy health burden and increased healthcare costs. The incidince of nosocomial diarrhoea, *C. difficile*-associated nosocomial diarrhoea rates and the resistance status of this nosocomial pathogen are not clear in our country.

Objectives

To determine the *C. difficile*-associated nosocomial diarrhoea incidence and to review the current resistance status of *C. difficile* were the major aims of the present study.

Methods

This prospective clinical study included 100 diarrhoea samples from hospitalized patients in İstanbul, Turkey. The diarrhoea samples were investigated by culture methods via *Clostridium difficile* selective agar and bacterial resistance profiles were calculated with the E-test method.

Conclusions

The *C. difficile* resistance rates were determined for metronidazole as 29.4%, for vancomycin and teikopilanin as 2.9%. Our findings corroborate the alarming reports about the increasing metronidazole resistance rates of *C. difficile*. Our results support that the *C. difficile* is still an important factor in nosocomial diarrhoea. Furthermore, highness of antibiotic resistance for metronidazole may be caused by difficulties in treatment. The results indicate the necessity of further studies to develop control measures and effective treatment options for patients.

FEMS7-1173
Environmental Microbiology/Microbial Ecology /Microbial Communities

DIVERSITY OF XANTHOMONAS ARBORICOLA PV. JUGLANDIS IN PORTUGAL EVOQUES A COSMOPOLITAN DISPERSION

P. Albuquerque[1], C. Fernandes[1,2,3], L. Cruz[3,4], F. Tavares[1,2]
[1]CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos- InBIO- Laboratório Associado- Universidade do Porto, MDE - Microbial Diversity and Evolution, Vairão, Portugal
[2]FCUP – Faculdade de Ciências- Universidade do Porto- Rua do Campo Alegre s/nº, Departamento de Biologia, Porto, Portugal
[3]INIAV - Instituto Nacional de Investigação Agrária e Veterinária- Av. da República- Quinta do Marquês, UEIS-
SAFSV - Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal, Oeiras, Portugal
[4]Bioisi - Biosystems & Integrative Sciences Institute- Campo Grande,
Faculdade de Ciências- Universidade Lisboa, Lisboa, Portugal

Backgrounds

Xanthomonas arboricola pv. *juglandis* (*Xaj*) is the most important phytopathogenic bacteria affecting walnut-growing areas worldwide. Serious concerns about its economic impact made *Xaj* the focus of several epidemiological studies. Regardless the genetic diversity of *Xaj* reported to date, further studies are needed to fully characterize the diversity of *Xaj* and determine its significance.

Objectives

This study aimed to unveil the genetic diversity of Portuguese *Xaj* populations, and determine possible correlations between distinct *Xaj* clonal lineages and: walnut cultivars; bioclimatic factors; virulence fitness in leaves and fruits; and co-infection potential.

Methods

One hundred and seven *Xaj* isolates were obtained from leaves, fruits, branches, catkins and buds of symptomatic walnut trees distributed throughout distinct climatic regions of Portugal. Isolates' diversity was assessed using a dot blot hybridization platform with nine *Xaj*-specific DNA markers and by MLSA, using four housekeeping genes (*acnB*, *fyuA*, *gyrB* and *rpoD*).

Conclusions

MLSA allowed to verify that Portuguese *Xaj* lineages are genetically heterogeneous, comprised of at least eight different clusters. Such diversity was further sustained by the sixteen different hybridization patterns obtained. A cluster of six isolates was shown to diverge from the main lineages of the other *Xaj* characterized in this work or other *X. arboricola* strains previously described. Furthermore, the present study suggested that *Xaj* variability is not dependent on geographical areas, walnut cultivars, plant organ or isolation date, which is evocative of a cosmopolitan dispersion. Interestingly, the results highlighted the presence of different lineages within the same walnut host tree.

FEMS7-1089
Environmental Microbiology/Microbial Ecology /Microbial Communities

OPTIMISATION OF 16S RRNA GUT MICROBIOTA PROFILING OF EXTREMELY LOW BIRTH WEIGHT INFANTS

C. Alcon[1], S. Mitra[2], J. Ketskemety[1], U. Wegmann[1], J. Wain[3], G. Belteki[4], P. Clarke[5], L. Hall[1]
[1]Institute of Food Research, Gut and Health, Norwich, United Kingdom
[2]Leeds Institute for Biomedical and Clinical Sciences,
Leeds Institute for Biomedical and Clinical Sciences, Leeds, United Kingdom
[3]University of East Anglia, Norwich Medical School, Norwich, United Kingdom
[4]The Rosie Hospital, Neonatal Instensive Care Unit, Cambridge, United Kingdom
[5]Norfolk and Norwich University Hospital, Neonatal Intensive Care Unit, Norwich, United Kingdom

Backgrounds

Preterm birth infants, particularly those born with an extremely low weight (<1kg), may have an altered gut microbiota due to disturbances induced by factors including; gut immaturity, delivery mode and antibiotic treatments. These microbiota disturbances may increase the risk of certain preterm-associated diseases such as necrotising enterocolitis. Therefore there is a requirement to optimally characterise gut microbial profiles in this at-risk cohort, particularly for studying the influence of microbiota therapies (e.g. probiotic supplementation) on microbial profiles and health outcomes.

Objectives

NA

Methods

Next generation sequencing targeting the bacterial 16S rRNA region allows in-depth and high-throughput characterisation of complex microbial communities. However, previous studies using adult samples indicate DNA extraction and the hypervariable region targeted can significantly impact the representative microbiota profile. We have optimised a protocol for studying the composition of the very early life gut microbiota, with a particular focus on the beneficial bacterial genus Bifidobacterium. Our study comprises Extremely Low Birth Weight (ELBW) infants (<27 week's gestation with/without probiotic supplementation), and control term infants. We compared three different DNA extraction methods, and subsequently PCR amplified and sequenced three hypervariable regions of the 16S rRNA gene (V1+V2+V3), (V4+V5) and (V6+V7+V8), which we compared to paired shotgun metagenomic data.

Conclusions

Our data indicate that all steps of the 16s microbiota profiling pipeline significant effect the microbiota profiles obtained from ELBW. Thus, it is important to carefully optimise 16s protocols for specific patient cohorts as different methodologies may lead to misrepresentation of profiles and overall conclusions about the impact of different interventions.

FEMS7-2237
Environmental Microbiology/Microbial Ecology /Microbial Communities

RESPONSES OF SOIL BACTERIA COMMUNITIES TO VARIOUS HYDROCARBON CONTAMINANTS

H. Aldosari[1], *A. Grant*[1]
[1]*University of East Anglia, School of Environmental Sciences, Norwich, United Kingdom*

Backgrounds

Hydrocarbons contamination of soil is a widespread and urgent environmental problem. Many studies have examined impacts of a single crude or fuel oil on microbial community composition. Other studies have examined effects of one or more polycyclic aromatic hydrocarbons (PAHs), but rarely at the same time as crude or fuel oil.

Objectives

In consequence we do not know which components of oil have the biggest effects on microbial communities in the soil.

Methods

We compare responses of bacteria communities to a linear alkane (hexadecane), a PAH (fluoranthene) and diesel oil in different soil types, using Illumina sequencing of PCR amplified bacterial 16S sequences supplemented with metagenomics data.

Conclusions

There were significant impacts of all hydrocarbons on the communities in both soil types, with fluoranthene having the greatest effect. The extent to which the responses of communities in the two soils are similar depends upon the taxonomic level at which the comparison is made, helping to explain reports in the literature that different soils show idiosyncratic responses to hydrocarbon contamination.

FEMS7-1397
Environmental Microbiology/Microbial Ecology /Microbial Communities

GROWTH IN BIOFILM ENHANCES THE POTENTIAL TO FORM NEW BIOFILM IN CLINICAL ISOLATES OF KLEBSIELLA PNEUMONIAE

A. Hossain[1], M. Alharbi[2], A. Alateeq[2], A. Alatig[2], I. Alolayt[2], A. Alsayegh[2]
[1]University Of Hail, Microbiology, Hail, Saudi Arabia
[2]University Of Hail, Medicine, HAIL, Saudi Arabia

Backgrounds

Production of biofilm correlates with virulence as bacteria in biofilm often exhibit enhanced resistance to antibiotics and reduced clearance by immune system. Biofilm production potential of fresh clinical isolates of *Klebsiella pneumoniae* was investigated in this study emphasizing on whether growth in biofilm modulates the potential of the strains to form new biofilm and whether presence of normal human serum (NHS) in growth medium affects biofilm production potential.

Objectives

To determine whether growth in biofilm enhances the potential of new biofilm formation by *K. pneumoniae*.

To determine the influnece of normal human serum on biofilm forming potential of *K. pneumoniae* strains.

Methods

K. pneumoniae strains were obtained from the King Khaled Hospital, Hail. Saudi Arabia. Trypticase soy broth (TSB) was used for bacterial growth. Bacterial samples taken from biofilm or planktonic culture were used to initiate the formation of new biofilm for comparison. Crystal violet dye binding spectrophotometric assay was used for quantitation of biofilm. TSB was supplemented with 20% normal human serum (NHS) was used to determine whether NHS influences biofilm production.

Conclusions

The results of this study show that growth in biofilm enhances the potential to form new biofilm and production of biofilm was increased in presence of NHS.

FEMS7-0701
Environmental Microbiology/Microbial Ecology /Microbial Communities

BACTERIOPHAGES TO INCREASE THE SUSCEPTIBILITY OF A PATHOGENIC ANTIBIOTIC RESISTANT ESCHERICHIA COLI IN PRESENCE OF SOLAR DISINFECTION

N. Al-Jassim[1], D. Mantilla-Calderon[1], P. Hong[1]
[1]King Abdullah University of Science & Technology KAUST,
Environmental Science and Engineering- Division of Biological and Environmental Science and Engin eering, Thuwal Jeddah, Saudi Arabia

Backgrounds

Antibiotic-resistant bacteria (ARB) are emerging contaminants of growing concern. Our previous work characterized a pathogenic carbapenem-resistant *E. coli*, named PI-7, from the influent of a local wastewater treatment plant (Mantilla *et al*, 2016). To assess its fate/persistence upon release into the environment, its inactivation under simulated solar irradiance was examined. *E. coli* PI-7 underwent a 6-h lag-phase without decay, and took >12 h to achieve 5-log inactivation (decay half-life 2.85±0.46 min) (Al-Jassim *et al*, submitted). A persisting fraction remained detectable at 11-17 h, decaying almost three times slower (half-life 7.76±1.27 min).

Objectives

The prolonged persistence of this pathogenic ARB means that it is important to explore biocontrol strategies to attenuate its propagation. The current work explores using bacteriophages to increase the susceptibility of *E. coli* PI-7 upon solar inactivation, as gene-expression analysis on PI-7 revealed downregulation of phage-shock functions under simulated solar irradiance.

Methods

Seven bacteriophage isolates targeting PI-7 were isolated from wastewater influent, and checked for their lytic-specificity. Then, three bacteriophage isolates that showed the best stability under simulated solar irradiance conditions were selected for further trials with *E. coli* PI-7.

Conclusions

Bacteriophages showed lytic activity only against *E. coli* PI-7 and no other tested strains of *E. coli* and non-*E.coli* isolates. Under simulated solar irradiance, the selected bacteriophages significantly reduced *E. coli* PI-7 lag-phase length from 6.64±0.63 h to <1-4 h ($p \leq 0.0012$) compared to controls. These preliminary results show potential of bacteriophages as biocontrol agents that can be complementarily used with solar disinfection to prevent dissemination of *E. coli* PI-7.

References

Al-Jassim, Nada, et al. “Inactivation of a virulent wastewater *Escherichia coli* and non-virulent commensal *Escherichia coli* DSM1103 strains and their gene expression upon solar irradiation” *ES&T*. Submitted.

Mantilla-Calderon, David, et al. "Isolation and characterization of NDM-positive *Escherichia coli* from municipal wastewater in Jeddah, Saudi Arabia." *Antimicrobial Agents and Chemotherapy* 60.9 (2016):5223-5231.

FEMS7-0506
Environmental Microbiology/Microbial Ecology /Microbial Communities

BACTERIAL DIVERSITY ANALYSIS IN VINEYARD SOILS FROM RIBERA DEL DUERO

A. Alonso[1], J. García-Ruiz[1], P. García-Gonzalo[2], M.C. Lobo[2], E. Navascués[3], A. Santos[1], D. Marquina[1]
[1]Complutense University of Madrid, Microbiology III, Madrid, Spain
[2]IMIDRA, Agri-environmental research, Alcalá de Henares, Spain
[3]Pago de Carraovejas S.L., Research & Development, Peñafiel, Spain

Backgrounds

Vineyard soils are poorer in nutrients (potassium, iron, boron, manganese, copper and zinc) than other agricultural soils. Other environments showing high biodiversity indexes, such as reefs or jungle, are also poor in nutrients. Vineyards, usually distinguished for their antiquity and their poor nutrient status, tend to have ecological resilience and thus biodiversity stability.

Objectives

This project aims to determine the biodiversity (both culturable and non-culturable bacteria), associated to different vineyard soils from Ribera del Duero D.O., estimating the metabolic state of that soils.

Methods

Soil samples were collected along the entire physiological cycle of vines (including dormancy, budbreak and harvest status). Soil samples were collected from 5–30 cm depth from twelve different vineyard blocks, air-dried and then passed through a 2 mm sieve.

Culturable heterotrophic bacteria were estimated by count on TSA plates. In addition, *Actinomyces* species and nitrogen-fixing bacteria populations were estimated by culturing on appropriate selective media. Substrate Inducted Respiration (SIR) was used to determinate the oxidative metabolism of heterotrophic microorganisms present in soils. Edaphic bacterial biodiversity was determined by Denaturing Gradient Gel Electrophoresis (DGGE) using 70%-30% urea/formamide.

Conclusions

In dormancy and budbreak states, Shannon-Wiener index keeps on the highest values. SIR assay is a reliable method to estimate the non-culturable fraction of soil population, by comparing with total cell counts on TSA plates.

A population bloom in nitrogen-fixing bacteria occurs during budbreak, however it does not modify biodiversity index. Contrary, during harvest, bacterial biodiversity indexes decrease since a significant growth of *Actinomyces* spp. population (over 52%) is observed.

FEMS7-2630
Environmental Microbiology/Microbial Ecology /Microbial Communities

ISOLATION OF INDIGENOUS HYDROCARBON TRANSFORMING BACTERIA FROM OIL CONTAMINATED SOILS IN LIBYA: SELECTION FOR USE AS POTENTIAL INOCULA FOR SOIL BIOREMEDIATION

H. Althalb[1], *I. Elmisurati*[1]
[1]*Libyan Petroleum Institute, Industrial & Production Chemistry, Tripoli, Libya*

Backgrounds

Abstract The Libyan oil industry has left a significant legacy of contamination and methods are required to remediate oil- contaminated soils in the area.

Objectives

In this work hydrocarbon utilizing microorganisms were isolated and identified from contaminated soil samples obtained from an oil Refinery (Zawia, Libya).

Methods

After initial screening of eleven isolates capable of growth on hexadecane, the five most promising hydrocarbon–utilizing bacteria were isolated and tested for biosurfactant production and emulsification activity. They were identified (using 16S rRNA sequence analysis) as *Pseudomonas putida*, *Pseudomonas species, Betaproteobacterium, Actinomyces species*, and *Bacillus species*.

Conclusions

Among the five species tested, *Pseudomonas putida* showed superior performance in terms of growth on hydrocarbons (1.0×10^{10} CFU (ml)), E24 emulsifying activity (86%) and ability to transform hydrocarbons in pure culture. Interestingly, gas chromatographic analysis of crude oil treated with *P. putida* showed a decrease in heavy hydrocarbon fractions demonstrating a clear potential for this microbe to be used as a soil inoculant in bioremediation.

FEMS7-0342
Environmental Microbiology/Microbial Ecology /Microbial Communities

FROM PAULOVIAN TO MICROBIAL CONDITIONING: MODULATION OF S. AUREUS QUORUM SENSING BY P. AERUGINOSA

C. Amador[1], *L. Jelsbak*[1]
[1]*Technical University of Denmark, Department of Biotechnology and Biomedicine, Kgs. Lyngby, Denmark*

Backgrounds

Pseudomonas aeruginosa and *Staphylococcus aureus* are pathogens frequently co-isolated in cystic fibrosis (CF) airway infections. We have previously determined the genetic basis of adaptation in the transmissible, CF-adapted *P. aeruginosa* DK2 lineage (PADK2).

Objectives

We aimed at elucidating whether and how the genetic changes in PADK2 may have re-modelled its ability to interact with other pathogenic bacteria, such as *S. aureus* (SA).

Methods

Infecting bacteria can grow as aggregates on the airway surfaces, hence we examined the *in vitro* transcriptional pattern of mono-culture or co-culture spots grown on LB agar. RNA-sequencing was performed for all samples, a pairwise analysis conducted for each strain and data validated by RT-qPCR and transcriptional fusions.

Conclusions

In co-culture, SA showed a general tendency of gene expression down-regulation, including many genes encoding virulence factors and regulated by the Agr Quorum Sensing (QS) system such as proteases, leukocidins, leukotoxins and haemolysins. Remarkably, the *agrD* gene encoding the precursor of the auto-inducing peptide – AIP – regulating QS, was also down-regulated in co-culture, pointing to inhibition of the Agr QS system. Conversely, PADK2 showed up-regulation of the *pqsD* gene – involved in biosynthesis of the QS molecule HHQ and a general pattern of enhanced gene expression.

Our *in vitro* approach evidences that interspecies interactions can alter pathogen behaviour and – in the case of *S. aureus* – results in a phenotype compatible with a persistent infection and enhanced survival in the host. The mechanism exerted by *P. aeruginosa* DK2 for down-regulation of *S. aureus* QS is however yet to be identified.

FEMS7-0425
Environmental Microbiology/Microbial Ecology /Microbial Communities

PROTECTOR AND BENEFACTOR FOR PLANTS: ACC DEAMINASE POSITIVE BACTERIA IN STRESSED ENVIRONMENTS

G. Anand[1], V.S. Bisaria[1], S. Sharma[1]
[1]Indian Institute of Technology, Department of Biochemical Engineering & Biotechnology, NEW DELHI, India

Backgrounds

Agriculture is under tremendous pressure worldwide to feed an ever increasing population. The misuse of chemicals as fertilizers and pesticides for increasing productivity has led to ecological and human health hazards. Bioinoculants as plant growth promoting rhizobacteria (PGPR) present an eco-friendly, long term and sustainable alternative to these chemicals. ACC deaminase protects the plant from all biotic and abiotic stresses by degrading the phytotoxic levels of ethylene produced when a plant encounters any stress. Also the diversity of ACC deaminase producers in the rhizosphere plays an important factor in amelioration of stress. Salinity and drought are the major factors impacting Indian agriculture and worldwide.

Objectives

The objectives were (i) diversity analysis of ACC deaminase producers in stressed agricultural environments to derive a correlation between the two, and (ii) enumeration and isolation of potent ACC deaminase producers along with other desirable PGP properties for amelioration of stress in fields.

Methods

Plant growth experiment was setup in natural conditions using *Cajanus cajan* UPAS 120 as the model crop with simulated salinity and drought conditions.

Conclusions

High salt and natural drought conditions increased the abundance of probable ACC deaminase positive bacteria. Isolates obtained were screened for other PGP properties; subsequently plant growth experiment was set up in controlled condition for validation. Extensive analysis of plant parameters responsive to stress were measured. The plants with bioinoculants performed better in stressed conditions than the untreated control. ACC deaminase producers was found to serve dual function: protecting the plant from stress conditions and also promoting plant growth and productivity.

FEMS7-2074
Environmental Microbiology/Microbial Ecology /Microbial Communities

ANALYSIS OF THE BIOFILM FORMATION IN A HYDROTHERMAL SPRING CAVE USING IN SITU EXPERIMENTAL MODEL SYSTEM

D. Anda[1], J. Makk[1], A. Szabó[1], P. Bodor[2], J. Mádl-Szőnyi[2], K. Márialigeti[1], A. Borsodi[1]
[1]Eotvos Lorand University, Department of Microbiology, Budapest, Hungary
[2]Eotvos Lorand University, Department of Physical and Applied Geology, Budapest, Hungary

Backgrounds

The Buda Thermal Karst System (Budapest, Hungary) is one of the few hydrogeological systems, where springs, caves and the effects of hydrothermal fluids on carbonates can be evaluated. The number of microbiological studies related to the karst waters and biofilms developed on the surface of spring caves has been increased in the last decade in Hungary.

Objectives

However, the phases of biofilm formation and the influencing factors remained unknown. The Rudas-Török spring cave located in the southern part of Buda Thermal Karst System has optimal conditions as a study model.

Methods

For the evaluation of factors influencing biofilm formation, glass sides were immersed into the water-filled cave, and samples were taken at three weekly intervals during the 30 weeks of the monitoring phase. Besides field measurements and microscopic (light microscopy and FIB-SEM) techniques applied for the determination of physical and chemical parameters; a pyrosequencing approach based on the variability of the bacterial 16S rRNA gene was used to reveal the characteristic taxa and major shifts in the structures of the developing bacterial community.

Conclusions

The experiment resulted in an increase in the taxonomic diversity of bacterial communities which reached the maximum at phylum level in the third week. The members of phyla Chloroflexi, Parcubacteria, Plantomycetes, Proteobacteria, WCHB1-60 and Nitrospirae proved to be most abundant but their relative abundance changed at different rates during the studied period. Furthermore, there were remarkable changes in the OTU composition during the development of the biofilm.

This research was supported by the Hungarian Scientific Research Fund (OTKA) Grant NK101356.

FEMS7-0577
Environmental Microbiology/Microbial Ecology /Microbial Communities

SHIFT OF MICROBIAL COMMUNITY COMPOSITIONS IN DENTAL BIOFILMS AND CONSEQUENTIAL MINERAL CHANGES OF DENTAL ENAMEL FOLLOWING DIETARY CHANGES

A.C. Anderson[1], M. Rothballer[2], M.J. Altenburger[1], L. Karygianni[1], J.P. Wölber[1], E. Hellwig[1], M. Schmid[2], A. Al-Ahmad[1]
[1]Albert-Ludwigs University Freiburg, Department of Operative Dentistry and Periodontology, Freiburg, Germany
[2]Helmholtz Center- German Research Center for Environmental Health, Research Unit Microbe-Plant Interactions, Munich, Germany

Backgrounds

Caries development is associated with a shift in microbial communities of dental biofilms. Increasing numbers of acidogenic bacterial species, due to frequent simple carbohydrate consumption, result in cariogenic plaque.

Objectives

Few studies have analyzed environmentally induced shifts in oral biofilms *in situ*. Here the influence of a defined dietary change on the microbial community has been studied.

Methods

Eleven individuals wore splint systems containing bovine enamel slabs for 3x7 days with 7-day intervals to obtain dental biofilm samples, while keeping their regular diet. Subsequently, individuals sucked rock candy 5x per day for 3 months. Biofilm samples were collected at the end of this phase. The regular diet of the individuals was monitored. The microbial community of the dental biofilm was analyzed using Illumina MiSeq sequencing, amplifying variable regions v1-v2. Streptococci sequences were manually analyzed to the species level using ARB. Changes in mineral loss and lesion depth of the bovine enamel were investigated using transverse microradiography (TMR).

Conclusions

Permanova analysis revealed a significant difference in beta-diversity in the two phases. The individual dental biofilm communities were dominated in both phases by oral streptococci with a highly significant increase of *Streptococcus gordonii* ($p \leq 0.001$) and significant increases of *Streptococcus parasanguinis* ($p=0.04$) and *Streptococcus sanguinis* ($p=0.01$) in phase II. Simultaneously TMR showed significant demineralization of dental enamel ($p=0.0095$).
Our results showed that the prevalence of streptococci other than mutans streptococci correlates with enhanced enamel demineralization due to high acid production, following elevated simple carbohydrate consumption, supporting the extended ecological plaque hypothesis.

FEMS7-3216
Environmental Microbiology/Microbial Ecology /Microbial Communities

INFLUENCE OF SOLAR RADIATION AFTER MECHANICAL REVOLVING ON THE MICROBIOLOGICAL QUALITY OF THE DRY SANDS OF COASTAL BEACHES STATE OF PARANA-BRAZIL

S. Andraus[2], J. Alves Dionisio[1]
[1]Universidade Federal Do Parana, Solos E Engenharia Agricola, Curitiba, Brazil
[2]Instituto Ambiental do Paraná, Curitiba, Brazil

Backgrounds

Microbiological contamination of beach sand is a global reality which represents a potential for transmitting diseases.

Objectives

The purpose of the study, which is being carried out over the last four years, is to improve the sanitary aspects of the sands of the beaches of Paraná, by cleaning and revolving the organic debris and thus contribute to the reduction of diseases transmitted by direct contact.

Methods

During the study period, from January 2016 to February 2017, 15 samples were taken on three beaches along the coast of Paraná (Caiobá, Matinhos and Guaratuba), which have a high concentration of bathers, mainly in the summer. Two points were selected by beach, demarcated with an area of 50 m^2 (5 m x 10 m) and the dry sand samples were collected manually, of the superficial layer of approximately 10 cm. For the accomplishment of the microbiological analyzes, the enzymatic system fluorogenic and chromogenic substrate was used to isolate the Escherichia coli and the chromogenic substrate for Enterococcus.

Conclusions

The results of the sand analyzes, regarding the monitoring carried out from January 2016 to February 2017, using the indicators Escherichia coli and Enterococos, show a significant decrease in the summer seasons of 2015-2016 and 2016-2017. The results can be attributed to the action of solar rays, such as ultraviolet, on the surface of dry sands, reducing the action and eliminating pathogens, responsible for numerous diseases, acquired by direct contact.

FEMS7-0594
Environmental Microbiology/Microbial Ecology /Microbial Communities

TOP-DOWN REGULATION OF PROKARYOTIC GROWTH EFFICIENCY IN TEMPERATE FRESHWATER PELAGIC ENVIRONMENTS

A.S. Pradeep Ram[1], J. Colombet[1], A. Thouvenot[2], T. Sime Ngando[1]
[1]Université Clermont Auvergne, LMGE- UMR CNRS 6023, AUBIERE, France
[2]Université Clermont Auvergne, Athos Environnement, AUBIERE, France

Backgrounds

In aquatic systems, limited data exists on the impact of mortality forces such as viral lysis and flagellate grazing when seeking to explain factors regulating prokaryotic metabolism.

Objectives

We explored the relative influence of top-down factors (viral lysis and heterotrophic nanoflagellate grazing) on prokaryotic mortality and their subsequent impact on their community metabolism in the euphotic zone of 21 temperate freshwater lakes located in the French Massif Central.

Methods

Viral lysis was determined from frequency of visibly infected prokaryotic cells by transmission electron microscopy whereas heterotrophic nanoflagellate grazing potential was calculated from flagellate clearance rate estimates.

Conclusions

Prokaryotic growth efficiency (PGE, index of prokaryotic community metabolism) determined from prokaryotic production and respiration measurements varied from 5 to 74% across the lakes. Viral and potential grazer induced mortality of prokaryotes had contrasting impact on PGE. Potential flagellate grazing was found to enhance PGE whereas viral lysis had antagonistic impacts on PGE. The average PGE value in the grazing and viral lysis dominated lake water samples was 35.4% (± 15.2%) and 17.2% (± 8.1%) respectively. Selective viral lysis or flagellate grazing on prokaryotes together with the nature of contrasted substrates released through mortality processes can perhaps explain for the observed variation and differences in PGE among the studied lakes. The influences of such specific top-down processes on PGE can have strong implications on the carbon and nutrient fluxes in freshwater pelagic environments.

FEMS7-0620
Environmental Microbiology/Microbial Ecology /Microbial Communities

CHARACTERISING THE MICROBIAL COMMUNITIES ASSOCIATED WITH THE WATER DISTRIBUTION SYSTEM OF A BROILER FARM AND THEIR ROLE IN CAMPYLOBACTER INFECTION

P. Aranega Bou[1], *I. Goodhead*[1], *R. Birtles*[1], *C. James*[1]
[1]*University of Salford, School of Environment and Life Sciences, Salford, United Kingdom*

Backgrounds

Campylobacter is the most frequent food-borne illness in the EU with 190,000 cases reported annually. Poultry is an important reservoir for this zoonosis and the handling and consumption of chicken meat may directly account for 20-30% of cases. Despite the large body of research, sources of on-farm contamination remain unidentified. It has been suggested that natural microbial communities may influence *Campylobacter* survival in environmental niches.

Objectives

This study characterises the microbial communities that inhabit the broiler farm water system of a commercial farm in UK and how it changes over the 7-week production cycle and correlates with the presence of *Campylobacter*.

Methods

DNA was extracted from weekly biofilm and bulk water samples for 16S and 18S amplicon profiling. In parallel, selective media and molecular techniques were used to detect *Campylobacter* ssp.

Conclusions

Microbial communities were shown to vary significantly across different sample types (biofilm vs. bulk water and broiler farm vs. source water) and also temporally. Broiler house bacterial communities were dominated by Proteobacteria with a shift towards Firmicutes mainly due to an increase in *Lactobacillus* and *Staphylococcus* towards the end of the cycle. *Campylobacter*-specific 16S rRNA reads were detected in weeks 5 and 7 suggesting a possible role of the water distribution system in *Campylobacter* transmission. However, no viable *Campylobacter* colonies were isolated, suggesting a viable but non-culturable state. The emerging pathogens *C. ureolyticus* and *Helicobacter pullorum* were also detected. Microbial profiling could provide clues about which farm conditions might promote or inhibit survival of *Campylobacter* and other pathogens.

FEMS7-2344
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECT OF ELECTRO FENTON TREATMENT ON MICROORGANISMS OF WASTEWATER TREATMENT PLANT EFFLUENTS

R.M. Araujo Boira[1], E. Anfruns-Estrada[1], H. Salvadó[2], C. Bruguera-Casamada[1], I. Sirés[3], E. Brillas[3]
[1]Universitat de Barcelona, Genetica- Microbiologia i Estadistica, Barcelona, Spain
[2]Universitat de Barcelona, Department de Biologia Evolutiva- Ecologia i Ciencies Ambientals, Barcelona, Spain
[3]Universitat de Barcelona,
Laboratori d'Electroquímica dels Materials i del Medi Ambient- Departament de Química Física, Barcelona, Spain

Backgrounds

Electrochemical advanced oxidation processes (EAOPs) have been gaining increasing interest for wastewater decontamination. Conversely, much less attention has been paid to their ability for water disinfection. One of these processes is electro-Fenton (EF), which involves the use of an anode like boron-doped diamond (BDD) and an air-diffusion cathode to produce H_2O_2 on site. Fe^{2+} is added as catalyst, yielding various reactive oxygen species that act as oxidants. Among them, hydroxyl radicals can potentially inactivate microorganisms, including pathogens and, furthermore, are able to gradually destroy the organic matter.

Objectives

The main goal of this work was to study the performance of EF for the inactivation of the microorganisms contained in treated urban wastewater.

Methods

Microorganisms decays represented as log (N_t/N_0) reductions were tested at different times for 30 min during EF treatment of a primary and a secondary effluent obtained from a wastewater treatment plant (WWTP) located near Barcelona. Four bacteria and one bacteriophage were assayed by culture and active eukaryote community was analyzed by microscopy.

Conclusions

Important microbial reductions were obtained using EF in both wastewater effluents after 30 min. Heterotrophic bacteria showed a decay of 4.1 log units in primary effluent and 3.2 log units in secondary effluent. *Escherichia coli*, *Enterococcus* sp. and Somatic Bacteriophages were rapidly inactivated, whereas protozoa were severely reduced in 10 min. In contrast, spores of *Clostridium perfringens* were the most resistant. In summary, EF is able to inactivate microorganisms with high efficacy and could be further explored as wastewater treatment.

FEMS7-1931
Environmental Microbiology/Microbial Ecology /Microbial Communities

THERMOHALOPHILIC BACTERIAL STRAINS ISOLATED FROM PETROLEUM RESERVOIR AND THEIR POTENTIALS FOR PRODUCTION OF SURFACE ACTIVE MOLECULES

M.N. Argentin[1], G.C. Costa[2], L.F. Martins[3], N.R.S. Bossolan[4]
[1]São Carlos Institute of Physics- University of São Paulo,
Graduate Program in Physics - Applied/Biomolecular, São Carlos, Brazil
[2]São Carlos Institute of Physics - University of São Paulo,
Graduate Program in Physics - Applied/Biomolecular, São Carlos, Brazil
[3]PETROBRAS, CENPES - Petrobras Research & Development Center, Rio de Janeiro, Brazil
[4]São Carlos Institute of Physics- University of São Paulo,
Department of Physics and Interdisciplinary Science, São Carlos, Brazil

Backgrounds

Biosurfactants (BS) have several industrial and environmental applications whose processes involve extremes of temperature, pressure, pH, and salinity, stimulating the research of microorganisms that produce BS under these conditions. In the petroleum industry, BS have potential to enhance the removal of oil from reservoirs, increasing oil production.

Objectives

To evaluate biosurfactant production by bacterial strains isolated from an oil reservoir, under conditions of high salinity and/or temperature, with a view to its application in tertiary oil recovery.

Methods

Strains were isolated from culture media inoculated with rock (strains Ar70C2, Ar35B3 and Ar70C7-2) and oil samples (strains O9-1 and O27-2) from an offshore Brazilian oil reservoir. Growth and BS production were measured from aerobic liquid cultures in LB broth, with salinity of 35g/L or 70g/L, without agitation, and incubation at 37^{o}C or 55^{o}C for 6-8 days. BS production was evaluated by E24 emulsification index and surface tension measurements. The identification of the isolates was done by sequencing the 16S rDNA gene.

Conclusions

The maximum E24 values recorded and the measured surface tension values were: 73% and 40.7mN/m for Ar70C2, 67% and 38.2 mN/m for Ar35B3, 70% and 46.1 mN/m for Ar70C7-2, 53% and 50.1 mN/m for O9-1, and 45% and 49.7mN/m for O27-2. Strain Ar70C2 was identified as *Pseudomonas libanensis* (96% similarity), Ar35B3 as *Melghirimyces thermohalophilus* (90%), Ar70C7-2 as *Bacillus alveayuensis* (99%), O9-1 as *Bacillus aquimaris* (99%), and O27-2 as *Bacillus subterraneus* (99%). All of these are described as species belonging to extreme environments in salinity and/or temperature, except *P.libanensis*.

FEMS7-1262
Environmental Microbiology/Microbial Ecology /Microbial Communities

RESISTOME OF CULTURABLE SOIL BACTERIA AND WHOLE SOIL OBTAINED FROM ECOLOGICAL AND INTENSE FARMING SITES

J. Armalyte[1], J. Skerniskyte[1], R. Juskaite[1], R. Krasauskas[1], M. Ruzauskas[2], E. Suziedeliene[1]
[1]Life Sciences Center- Vilnius University, Institute of Biosciences, Vilnius, Lithuania
[2]Lithuanian University of Health Sciences, Microbiology and Virology Institute, Kaunas, Lithuania

Backgrounds

The resistance of non-pathogenic soil microorganisms to modern day antibiotics has come into notice in the recent years. The ecology, evolution and development of natural resistome and its possible spread to humans and animals is an area of interest and concern.

Objectives

In this study we aimed to compare the resistomes of bacterial populations in various farming environments and distinguish the influence of human interference. This could help indicating the possible routes of antibiotic resistance gene dissemination.

Methods

MIC values of antibiotic resistant bacteria from ecological and intensive wheat farming fields (soil 1 and 2) were tested. Clinically important genes responsible for enzyme-mediated resistance to β-lactams (including 3th and 4th generation of cephalosporins, carbapenems), quinolones, and transferable genetic determinants integrons were investigated in culturable bacteria and total DNA extracted from the same soils.

Conclusions

Antibiotic-resistant culturable bacteria from both soils were mostly comprised of *Pseudomonas* genus. Most of the isolates were highly resistant to antibiotics, on the average to 8 and 11 antibiotics for soils 1 and 2, respectively. None of the isolates tested were positive for clinically important resistance genes. Similar results were observed when total soil DNA was screened. The only resistance genes detected in total soil DNA were bla_{TEM} and *tetM*. The mechanisms responsible for antibiotic resistance of soil bacteria differ from those observed in clinical settings and should be investigated as a potential source of new antibiotic resistance genes.

This research was funded by a grant (SIT-6/2015) from the Research Council of Lithuania

FEMS7-1274
Environmental Microbiology/Microbial Ecology /Microbial Communities

SEARCH FOR ANTIBIOTIC RESISTANCE MECHANISMS IN STENOTROPHOMONAS MALTOPHILIA RECOVERED FROM SOIL USING FUNCTIONAL DNA LIBRARIES

J. Armalyte[1], J. Skerniskyte[1], R. Krasauskas[1], M. Ruzauskas[2], E. Suziedeliene[1]
[1]Life Sciences Center- Vilnius University, Institute of Biosciences, Vilnius, Lithuania
[2]Lithuanian University of Health Sciences, Microbiology and Virology Institute, Kaunas, Lithuania

Backgrounds

Stenotrophomonas maltophilia is an ubiquitous environmental bacterium found in soil and water. It is also associated with an increasing number of nosocomial infections, affecting immuno-compromised patients. Treatment of this opportunistic pathogen is often difficult due to its intrinsic resistance to antibiotics.

Objectives

The resistance of *S. maltophilia* to antibiotics could be addressed to low membrane permeability, antibiotic modifying enzymes, multidrug efflux pumps, which functions often cannot be predicted from sequence information. In this work we aimed to distinguish genes responsible for antibiotic resistance of *S. maltophilia* using functional DNA fragment libraries.

Methods

Genomic DNA was isolated from 6 isolates of *S. maltophilia* recovered from wheat field, resistant to at least 2 classes of antibiotics. All the selected isolates showed resistance to imipenem. gDNA was fragmented and used to create functional DNA fragment library with fragment sizes of >3 kb. The library was transformed to *E. coli* and screened for antibiotic (β-lactams, aminoglycosides, quinolones) resistant clones.

Conclusions

Despite selected *S. maltophilia* soil isolates being resistant to β-lactams, aminoglycosides and quinolones, the search of resistant DNA fragment library clones only produced several clones of moderate imipenem resistance. None of the clones encoded β-lactamases, but one clone was found to contain major facilitator superfamily (MFS) transporter protein.

This research was funded by a grant (SIT-6/2015) from the Research Council of Lithuania

FEMS7-2299
Environmental Microbiology/Microbial Ecology /Microbial Communities

MOLECULAR CHARACTERISATION OF EXTENDED SPECTRUM BETA-LACTAMASES PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE

P. Montso[1], N.C. Ateba[1]
[1]North West University, Biological Sciences, Mafikeng, South Africa

Backgrounds

Extended Spectrum beta-lactamase producing *Enterobacteriaceae* particularly *Escherichia coli* and *Klebsiella pneumoniae* that possess ESBL traits cause hospital and community acquired infections worldwide.

Objectives

isolate and identify ESBL producing *Escherichia coli and Klebsiella pneumoniae* from cattle faeces and raw beef samples

Methods

Methods: A total of 151 samples were analysed and 259 presumptive isolates screened for characteristics of *Enterobacteriaceae* using biochemical tests and 16S rRNA universal gene. All the 259 isolates were subjected to *uidA*, *uspA* and *ntrA* specific PCR confirmed them as *E. coli* and *K. pneumoniae*. ESBL traits were detected on Brilliance ESBL agar and amplification of ESBL (bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{CMY-1}, bla_{CMY-2} and bla_{CTX-M}) gene determinants. Antimicrobial susceptibility test was performed to determine antibiotic resistance profiles of the isolates. Cluster analysis was performed using antibiotic inhibition zone diameter data to determine the relatedness between isolates from cattle faeces and beef samples. The genetic relatedness of all confirmed *E. coli* and *K. pneumoniae* was determined using ERIC-PCR analysis.

Results: A total of 114 and 82 *E. coli* and *K. pneumoniae* respectively were obtained. Large proportions (66.7-100%) of isolates were resistant to Amoxicillin, Aztreonam, Ceftazidime, Cefotaxime and Piperacillin. A total of 53.1% ESBL isolates were detected on Brilliance ESBL agar. The bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were detected in 85.5%, 69.6% and 58% of the isolates respectively. The bla_{CTX-M} and bla_{OXA} genes were dominant in *E. coli* and *K. pneumoniae* isolates respectively. Genetic fingerprints of *E. coli* and *K. pneumoniae* isolates were very similar.

Conclusions

Need for continuous monitoring of ESBL in animals.

FEMS7-2316
Environmental Microbiology/Microbial Ecology /Microbial Communities

MOLECULAR CHARACTERISATION OF VIRULENCE DETERMINANTS IN ESCHERICHIA COLI PATHOTYPES ISOLATED FROM ABATTOIRS

C. Tchatchouang[1], A. Kumar[1], C. Ateba[1]
[1]North West University, Biological Sciences, Mafikeng, South Africa

Backgrounds

Food products of animal origin particularly beef are known to cause *E. coli* related diseases in humans worldwide. *E. coli* strains are classified into different pathological groups (EPEC, EHEC, ETEC, EAEC, DAEC and EIEC) based on virulence characteristics and they can contaminate meat in abattoirs.

Objectives

isolate and characterize *E. coli* pathotypes from beef carcasses

Methods

Methods: A total of 2196 beef swab samples were collected, analysed on EMBA and 152 samples that were positive for *E. coli* based on macroscopic morphologies. 291 metallic sheen isolates were subjected to Gram-staining, oxidase, TSI, citrate utilization and *uidA* specific PCR analysis. *E. coli* isolates were screened for the presence of *stx1*, *stx2*, *eaeA* and *hlyA* virulence gene determinants. The combinations of the various virulence genes per isolate were used to determine their genotypes.

Results: A large proportion 152 (77.6%) of the samples were positive for *E. coli* macroscopically. A large proportion 256 (88 %) were positive for the *E. coli uidA* gene. Sixteen major genotypes designated G1 to G16 were identified and 199 (77.7%) isolates harboured the stx_1 gene while 70.7% to 77.7% harboured the stx_1, stx_2 and *eaeA* genes. Only 3 (1.2%) harboured both stx_1, and stx_2 genes (Genotype G6). Six (2.3%) isolates harboured all the four virulence gene determinants investigated while only 16 (6.3%) possessed the stx_1 and stx_2 genes respectively in combination with the two accessory virulence gene determinants (*eaeA* and *hlyA*) (G14, G15 and G16). ERIC fingerprints comprised 1 to 17 polymorphic bands per isolate.

Conclusions

Need for continuous monitoring

FEMS7-1212
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECTS OF NOVEL NANOCOMPOSITES ON MORPHOLOGICAL AND FUNCTIONAL PARAMETERS OF BACTERIA

A. Augustyniak[1], K. Cendrowski[2], P. Nawrotek[1], M. Barylak[2], E. Mijowska[2]
[1]West Pomeranian University of Technology- Szczecin- Faculty of Biotechnology and Animal Husban dry, Department of Immunology- Microbiology- and Physiological Chemistry, Szczecin, Poland
[2]West Pomeranian University of Technology- Szczecin- Institute of Chemical and Environment Engine ering, Department of Nanotechnology, Szczecin, Poland

Backgrounds

Novel nanostructures of various applications are being synthetized every year. Nevertheless, these substances may enter natural environment where their activity has not been fully understood. First organisms that will likely come into contact with nanostructures are environmental bacteria. Therefore, there is a necessity to evaluate the impact of nanostructures on these microorganisms.

Objectives

The goal of study was the evaluation of selected morphological and functional parameters of model and environmental bacteria in reaction to silica-based (nanospheres and nanotubes) and carbon-based (carbon nanotubes, and graphene oxide nanoflakes) nanomaterials functionalised with metals or titanium dioxide, and a comparison of the results between microorganisms.

Methods

Studies were conducted on selected bacteria from genera *Escherichia*, *Pseudomonas*, *Staphylococcus*, and *Streptomyces*. Nanocomposites selected for the experiments included silica nanospheres and nanotubes, carbon nanotubes, and graphene oxide flakes, modified with copper, cobalt, or titanium dioxide. Morphology and viability was evaluated by culture methods, phase-contrast microscopy, scanning and transmission electron microscopy. Metabolic activity was measured in the alamarBlue® assay. Secretion of pigments was measured by UV-Vis spectroscopy. Biofilm formation was evaluated in method based on staining with crystal violet. Absorbed nanostructures were detected by energy dispersive X-ray spectroscopy. Expression of selected genes was measured in Two-Step RT-qPCR reactions.

Conclusions

Environmental bacteria (streptomycetes and pseudomonads) show different reaction to nanocomposites than model strains (*Escherichia*, *Staphylococcus*). Nanocomposites may induce rapid clustering behaviour and secretion of secondary metabolites. Influence on biofilm formation is dependent on the type of nanomaterial and microorganism, and may be either induced or inhibited.

FEMS7-1226
Environmental Microbiology/Microbial Ecology /Microbial Communities

ISOLATION AND CHARACTERISATION OF FIFTY STREPTOMYCETES ISOLATED FROM VARIOUS ENVIRONMENTAL SAMPLES

A. Augustyniak[1], B. Grygorcewicz[2], M. Roszak[1], J. Jabłońska[1], P. Nawrotek[1]
[1]West Pomeranian University of Technology- Szczecin- Faculty of Biotechnology and Animal Husbandry, Department of Immunology- Microbiology- and Physiological Chemistry, Szczecin, Poland
[2]West Pomeranian University of Technology- Szczecin Faculty of Biotechnology and Animal Husbandry, Department of Immunology- Microbiology- and Physiological Chemistry, Szczecin, Poland

Backgrounds

Streptomycetes are microorganisms that inhabit many terrestrial and aqueous ecosystems. These bacteria are known for their ability to produce many useful secondary metabolites including antibiotics, enzymes, and pigments, that is probably affected by the environment of their origin. Although intensive studies on genre *Streptomyces* has been conducted, every year brings novel outcome on features of these microorganisms. This encourages to gather collections of microorganisms that will be tested in direction of future application in biotechnological purposes.

Objectives

The aim of the study was to isolate and evaluate biotechnological potential of fifty distinguishable streptomycetes isolated from various environmental samples.

Methods

Isolation was conducted from soil samples collected in agricultural, industrial and environmentally protected areas, as well as from a swine slurry. Bacteria were isolated with the use of serial dilution method. Gained monocultures were evaluated in terms of lytic activity, pigment secretion, growth in high salt concentrations, and antagonistic activity against opportunistic pathogens including Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, and yeast (*Candida albicans*).

Conclusions

Results confirm that the source of isolation may affect the characteristics of streptomycetes. Moreover, majority of isolated streptomycetes show antagonistic activity against yeast, while only one strain was able to inhibit growth of *Pseudomonas aeruginosa*. Swine slurry may be considered as a source of streptomycetes of high proteolytic activity.

FEMS7-2199
Environmental Microbiology/Microbial Ecology /Microbial Communities

COMMUNITY ECOLOGY OF AEROBIC ANOXYGENIC PHOTOTROPHIC (AAP) BACTERIA AT VARIOUS TEMPORAL AND SPATIAL SCALES

A. Auladell[1], P. Sánchez[1], J.M. Gasol[1], I. Ferrera[1]
[1]Marine Sciences Institute - ICM-CSIC, Dept. of Marine Biology, Barcelona, Spain

Backgrounds

Aerobic anoxygenic phototrophs (AAPs) are photoheterotrophic microorganisms that require organic substrates for growth, but can derive a portion of their energy harvesting light using bacteriochlorophyll *a*. Their ecology, metabolism and physiology have been intensively studied during the last two decades, but there is still a lack of large-scale analyses needed to unravel their spatio-temporal dynamics.

Objectives

Here, we focus in the community ecology of AAPs by analyzing the diversity of their marker gene *pufM* in large metagenetic (PCR-amplification and Illumina sequencing) and metagenomic datasets from 2 global circumnavigation expeditions, Tara Oceans and Malaspina, as well as from a decade-long time-series in the NW Mediterranean Sea (Blanes Bay Microbial Observatory).

Methods

At the spatial scale, we observed that variation in community structure was driven both by geographical location and by environmental conditions. In most stations phylogroups associated with the *Gammaproteobacteria*, *Roseobacter* and *Rhodobacter* were dominant but, interestingly, in some stations half of the sequences could not be associated to any defined phylogroup. At temporal scales, seasonality clearly differentiated AAP communities and half of the variation was explained by day length, temperature, water transparency, chlorophyll *a*, salinity and phototrophic nanoflagellates abundance. Comparing spatial and temporal scales, we observed that although higher richness was observed at temporal scales in a single site, dissimilarities between samples at spatial scales presented more variation, which increased with geographical distance.

Conclusions

Our work shows that a combination of large genetic studies with global and eulerian sampling have the most powerful potential to discern the spatio-temporal dynamics of marine microbial communities.

FEMS7-0898
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFICIENCY OF DRILLING FLUID ISOLATED BACTERIA ON CYANIDE TREATMENT

N.H. Avcioglu[1], *S. Bilen Ozyurek*[1], *I. Seyis Bilkay*[1]
[1]*Hacettepe University Faculty Of Science, Biology Biotechnology Section, Ankara, Turkey*

Backgrounds

Anthropogenic effects and industrial processes are the main sources of cyanide and cyanogen compounds which are known as important toxic chemicals for living organisms. Accordingly, by means of cyanide accumulation in environment, chemical composition of soil may change and it may also contaminate underground water and cause serious effects on aquatic organisms.

Objectives

It is very important to protect living organisms from the toxic effects of cyanide and cyanogen compounds. In that case, biological treatment methods are seen as advantageous for the removal of cyanide into nontoxic products. Accordingly, in biological treatment processes, lots of microorganisms and plants are being used in order to protect environment from the toxic, carcinogenic and mutagenic effects of cyanogen wastes.

Methods

In this study, cyanide removal efficiencies of *Proteus mirabilis*, *Klebsiella pneumoniae, Bacillus tequilensis, Bacillus axarguiensis* and *Enterobacter cloacae* which were isolated from drilling fluid, were investigated. Additionally, mix cultures were prepared and antagonistic and synergetic interactions between these bacterial species were also determined.

Conclusions

The results of this research demonstrated the usability of *Proteus mirabilis*, *Klebsiella pneumoniae, Bacillus tequilensis, Bacillus axarguiensis* and *Enterobacter cloacae* on cyanide treatment processes including different cyanogen wastes. Additionally, the importance of antagonistic and synergetic interactions between these microorganisms was also examined with this study. In this respect, the results are promising for the future researches on cyanide removal by using mix cultures of bacterial species.

FEMS7-0929
Environmental Microbiology/Microbial Ecology /Microbial Communities

INVESTIGATION OF CYANIDE TOLERANCE IN MICROCOCCUS LUTEUS

N.H. Avcioglu[1], S. Bilen ozyurek[1]
[1]Hacettepe University Faculty Of Science, Biology Biotechnology Section, Ankara, Turkey

Backgrounds

In parallel with the population increase all over the world, industrial production increases and high volumes of cyanide containing wastes originate from different industrial processes. Especially, untreated wastewaters discharged from these processes pollute the environment and cause toxic effects on cellular metabolism of living organisms.

Objectives

Removal of cyanide especially in industrial wastewaters by using effective methods is an important issue in order to protect the health of living organisms.Accordingly, wastewater management by means of using biological systems is environmentally friendly and new researches focus on the improvement of effective treatment methods by using microorganisms.

Methods

In this study, cyanide biodegradation capability of *Micrococcus luteus* against different cyanide compounds were investigated. Additionally, cyanogen waste forming industrial processes' wastewaters were selected and the usability of this strain in wastewater treatment was also investigated. Lastly, different concentrations of cyanide containing culture media were prepared and cyanide tolerance of *Micrococcus luteus* was also determined.

Conclusions

In the view of the results of this research, *Micrococcus luteus* is found as a usable agent in cyanide removal processes with its high cyanide tolerance ability.

FEMS7-2078
Environmental Microbiology/Microbial Ecology /Microbial Communities

AN IN VITRO STUDY OF HUMAN BACTERIAL COMMUNITY DYNAMICS AFTER FECAL TRANSPLANTATION

J. Arnold[1], I. Williamson[2], I. Carroll[1], J. Rawls[3], S. Magness[4], M.A. Azcarate-Peril[1]
[1]University of North Carolina at Chapel Hill,
Medicine – Division of Gastroenterology and Hepatology and Microbiome Core Facility- Center for Gastrointestinal Biology and Disease- University of North Carolina- Chapel Hill- NC, Chapell Hill, USA
[2]University of North Carolina at Chapel Hill, Biomedical Engineering, Chapell Hill, USA
[3]Duke University Medical Center, Department of Molecular Genetics & Microbiology, Durham, USA
[4]University of North Carolina at Chapel Hill, Cell Biology and Physiology and Biomedical Engineering, Chapell Hill, USA

Backgrounds

Fecal microbiota transplantation (FMT) treatments have been reported to successfully treat *Clostridium difficile* infections (CDI), and are currently considered for other dysfunctions like diabetes, IBD, IBS, metabolic syndrome, neurodevelopmental disorders, autoimmune diseases and allergic diseases. One drawback of FMT is the dependence on stool donors, and the potential unexpected consequences of using an undefined mixture of microbial species. Moreover, the impact of handling and processing of fecal samples on microbial viability and community dynamics has not been addressed.

Objectives

To monitor bacterial community dynamics of human stool samples post processing and FMT into stem cell-derived organoids.

Methods

We evaluated the impact of sample processing for FMT by 16S rRNA amplicon sequencing. Processed stool samples and pure bacterial cultures were inoculated into colonoids grown from conventionally raised mouse crypts. Plating, quantitative PCR, and 16S rRNA amplicon sequencing determined microbial survival, growth, and community dynamics inside the organoids. The impacts of antibiotics and oxygen exposure on live transplanted microbes were also evaluated.

Conclusions

Processing human fecal samples via homogenization and filtration did not significantly disrupt microbial diversity. Bacterial communities within the pseudo-lumen changed over 96 hours, showing an early bloom in Proteobacteria and subsequent recovery of Firmicutes. *Bifidobacterium adolescentis,* an obligate anaerobe, was able to survive and grow inside the organoid pseudo-lumen. Future studies will utilize this platform to determine impacts of microbiota colonization on cellular physiology, and how beneficial modulators of the microbiota effect microbial communities.

FEMS7-0708
Environmental Microbiology/Microbial Ecology /Microbial Communities

ENTERIC VIRUSES AMELIORATE GUT INFLAMMATION VIA TOLL-LIKE RECEPTOR 3 AND TOLL-LIKE RECEPTOR 7-MEDIATED INTERFERON-B PRODUCTION

J.W. Bae[1]
[1]*Kyung Hee University,*
Department of Life and Nanopharmaceutical Sciences and Department of Biology, SEOUL, Republic of Korea

Backgrounds

Metagenomic studies show that diverse resident viruses inhabit the healthy gut;

Objectives

however, little is known about the role of these viruses in the maintenance of gut homeostasis.

Methods

Mice deficient in both TLR3 and TLR7, which lack the ability to recognize viral single- and double-stranded RNAs, were more susceptible to DSS-induced experimental colitis. Furthermore, antiviral (AV) cocktail significantly decreased the abundance of enteric DNA and RNA viruses. To identify the genotypic changes of enteric DNA and RNA viruses, we conducted a metagenomic analysis of fecal VLP-derived metagenomic DNA and RNA using Illumina MiSeq sequencing.

Conclusions

In Jaccard and Hellinger distance-based principal coordinate analysis (PCoA), before and after paired samples of the VLP-derived metagenomes were distant from each other, and fecal VLP-derived DNA and RNA metagenomes were clustered by AV cocktail treatment ($P < 0.05$). Interestingly, we found an increase in the richness and abundance of *Caudovirales* bacteriophages in the enteric DNA virome of the mice treated with an AV cocktail, which corresponds with findings in a cohort of IBD patients. These results indicate that the genotypic changes in the community composition of enteric DNA and RNA viruses are implicated in deterioration against DSS-induced colitis by AV cocktail treatment. The recognition of resident viruses by TLR3 and TLR7 is required for protective immunity during gut inflammation.

FEMS7-1261
Environmental Microbiology/Microbial Ecology /Microbial Communities

DIFFERENTIAL GENE EXPRESSION OF VIBRIO TORANZONIAE IN RESPONSE TO ADVERSE CONDITIONS.

S. Balboa[1], A. Lasa[1], R. Jesús L.[1]
[1]CIBUS- Facultad de Biología. Universidade de Santiago de Compostela, Microbiology and Parasitology, Santiago de Compostela, Spain

Backgrounds

Viable but not culturable state (VBNC) can be defined as a dormancy phase in which bacterial cells maintain its viability but suffer major morphological and metabolic modifications becoming resistant to harsh conditions but losing culturability.

Objectives

To determine the capacity to enter in VBNC of environmental and pathogenic strains of *V. toranzoniae*.

Methods

Artificial seawater (15 ‰) microcosms were inoculated with the different bacterial strains and maintained at 4ºC. Expression of different genes was comparatively analysed by qPCR, among exponential growth, VBCN state and after resuscitation.

Conclusions

Environmental strain CECT 7225^T, entered in VBNC after 49 days of incubation and was resuscitated after 24h at 24ºC. However, the pathogenic strain R17 remained viable after 60 days, although bacterial counts dropped 4 log-units.

CECT 7225^T cells became coccoid after 24h. Concomitantly, the expression of the gene encoding for the cell shape protein, *mre*B, was reduced (2.5-fold) and recovered during the resuscitation although never reached the original levels. While in VBNC, cells were not able to divide, and thus, the *fts*Z expression was found to be reduced. The pathogenic strain R17 also showed rounded morphology and reduction of the *mre*B and *fts*Z expression after 50 days (8-fold). This results suggest that the morphology change is a response to the starvation rather than a VBNC characteristic.

The expression of the osmosensor *env*Z gene was up-regulated in strain CECT 7225^T during VBNC, while a dramatic down-regulation (5-fold) was found in starved R17 isolate, suggesting the importance of this sensor to trigger the entry in VBNC state.

FEMS7-1215
Environmental Microbiology/Microbial Ecology /Microbial Communities

CHARACTERIZATION OF THE AQUATIC MOBILOME IN A RIVER INFLUENCED BY A WASTEWATER TREATMENT PLANT

I. Lekunberri[1], J.L. Balcazar[1], C.M. Borrego[1,2]
[1]Catalan Institute for Water Research, Scientific and Technological Park of the University of Girona, Girona, Spain
[2]Group of Molecular Microbial Ecology, University of Girona, Girona, Spain

Backgrounds

Transfer of antibiotic resistance genes (ARGs) among bacteria through mobile genetic elements (MGEs), such as plasmids and bacteriophages, has been widely studied and demonstrated, especially in clinically relevant isolates. However, few studies have been undertaken to examine the role of MGEs in environmental settings.

Objectives

We investigated the abundance of ARGs in plasmid and phage DNA fractions from water samples collected upstream and downstream of the WWTP discharge point into the Ter River, Spain. The abundance of ARGs in bacterial DNA fractions was also analyzed for comparative purposes.

Methods

Real-time PCR assays were used to determine the abundance of genes encoding resistance to β-lactams, fluoroquinolones, tetracyclines, sulfonamides, glycopeptide antibiotics and macrolides. In the three DNA fractions, higher copy numbers of ARGs were detected in impact (after the WWTP effluent discharge) than in control sites, showing significant differences ($p < 0.05$) for most genes. Interestingly, genes conferring resistance to β-lactams (*bla*$_{TEM}$, *bla*$_{NDM}$ and *bla*$_{KPC}$) and vancomycin (*van*A) showed significant differences ($p < 0.05$) in DNA from MGEs (phages and plasmids) between control and impact sites but no statistical significance was obtained for these genes in the bacterial DNA fraction.

Conclusions

These results demonstrated that plasmids and phages play an important role in the mobilization of ARGs.

FEMS7-0886
Environmental Microbiology/Microbial Ecology /Microbial Communities

CONTRIBUTION OF FUNGI AND BACTERIA TO ECOSYSTEM PROCESSES IN FOREST SOILS: TRACING THE ACTIVITY OF COMMUNITIES AND INDIVIDUAL TAXA

P. Baldrian[1]
[1]*Institute of Microbiology of the CAS, Laboratory of Environmental Microbiology, Prague 4, Czech Republic*

Backgrounds

In forest soils, microbes are important drivers of soil processes, because they mediate decomposition as well as nutrient transfer from primary producers into soil.

Objectives

Here we have explored the involvement of microorganisms in ecosystem processes in coniferous forest topsoil.

Methods

Metatranscriptomics, metaproteomics and enzyme activity assays were combined with strain isolation and genome sequencing of major bacterial and fungal taxa to track microbial activity across seasons.

Conclusions

The results indicate that ecosystem processes exhibit seasonality with a fungal dominance in summer and bacterial dominance in winter affecting multiple processes ranging from ectomycorrhizal symbiosis to decomposition. Summer season is characterized as a period with rapid microbial growth accompanied by decomposition of recalcitrant plant biopolymers likely induced by the priming effect of photosynthates delivered by plant roots. Winter appears to be a period of slow growth when reserve compounds such as starch, glycogen and trehalose are utilized. Methods focusing environmental metacommunities are, however, not able to identify individual microbial species, participating in the soil processes. To do that, dominant bacterial strains were isolated and genome sequenced and genomes of ectomycorrhizal fungi were sequenced from fruitbodies. Mapping of metatranscriptomic reads on genomes of dominant bacteria showed, interestingly, that Acidobacteria are the likely major producers of decomposition-related enzymes that possess high counts of glycosyl hydrolases in their genomes, in contrast to Bacteroidetes and Proteobacteria. Transcript profiles of bacteria and fungi *in situ* differ among horizons as well as among seasons showing the effect of environmental conditions on transcription.

Žifčáková L et al. 2016. Environmental Microbiology 18:288.

FEMS7-0892
Environmental Microbiology/Microbial Ecology /Microbial Communities

CLEARCUTTING INITIATES COMPLEX RESTRUCTURALIZATION OF FUNGAL COMMUNITIES IN SOIL AND TREE ROOTS AND ALTERS DECOMPOSITION

M. Charvátová[1], P. Kohout[1], M. Šťursová[1], M. Tomšovský[2], P. Baldrian[1]
[1]Institute of Microbiology of the CAS, Laboratory of Environmental Microbiology, Prague 4, Czech Republic
[2]Mendel University in Brno, Faculty of Forestry and Wood Technology, Brno, Czech Republic

Backgrounds

Forest trees act as major suppliers of carbon into forest soils through their production of litter and rhizodeposition. Ectomycorrhizal fungi (ECM) produce their mycelia on expense of the rhizodeposited C and are thus dependent on their host.

Objectives

Here we followed fungal community composition in fine roots, rhizosphere soil and bulk soil of spruce (*Picea abies*) forest over two years following tree harvest.

Methods

Biomass and community analysis, enzyme assays.

Conclusions

Under living trees, rhizosphere contained significantly more fungal biomass than bulk soil and slightly less than roots. After tree harvest, fungal biomass in bulk soil remained unchanged, in the rhizosphere it decreased 4-fold while in decomposing roots it increased up to fivefold. This was associated with the increase in activity of decomposition-related enzymes in roots and decrease in rhizosphere. Fungal community composition changed over time. In the soil and rhizosphere, share of ECM decreased from 50% under living trees to <10% after two years. In roots of living trees, ECM represented 56% of all sequences and persisted until two months, but their abundance dropped to <2% after one year to be replaced by wood-decomposing taxa. Recently, the saprotrophic abilities of ECM are under debate and indirect evidences increase about the possible ability of certain taxa to decompose biopolymers, but although the persistence of ECM on dead roots was species dependent, our results do not support this view. Clearcutting induces sharp decrease in photosynthate allocation belowground and this is reflected by both the activity of soil microbiota and the composition of fungal communities.

FEMS7-1408
Environmental Microbiology/Microbial Ecology /Microbial Communities

EVALUATING THE USE OF MICROBIAL SOURCE TRACKING TECHNIQUES IN A MEDITERRANEAN CATCHMENT

E. Ballesté[1], C. García-Aljaro[1], M. Pascual-Benito[1], F. Lucena[1], A.R. Blanch[1]
[1]University of Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain

Backgrounds

Several host-specific markers to detect the source of faecal pollution in waterbodies have been designed and evaluated worldwide. These methods are mainly based in qPCR assays. They can be used for water management strategies and to model pollution events. However, geographical variability has been observed regarding the markers. Thus, the sensitivity and specificity has to be evaluated in the area of interest before their implementation and persistence studies have to be performed to model they fate in the environment.

Objectives

The aim of this study was to evaluate the sensitivity, specificity, and inactivation of 5 MST markers targeting human and animal pollution and finally model their fate in a river catchment.

Methods

The sensitivity and specificity of MST makers to detect human (HMBif and HF183), porcine (Pig2Bac), ruminant (BifCW) and poultry (BifPL) pollution was evaluated in 26 faecal samples collected in Spain. On the other hand, persistence analysis was performed onsite using dialysis bags. They were collected regularly during 2 weeks and inactivation of the MST markers was monitored. Finally the markers performance was evaluated in Riera de Cànoves after 8 sampling campaigns during one year. Samples were collected after the effluent of a WWTP, 500m and 1km downstream.

Conclusions

HMBif was the best option to determine human pollution with 100% sensitivity and 95% specificity. The sensitivity and specificity of animal markers was higher than 80% and 60%, respectively, being Pig2Bac the less specific. The inactivation of the markers was similar in summer (T_{90} between 1 – 2.2 days). On the other hand, in winter higher difference between them was observed (T_{90} between 2.1 - 6.9 days). Organic matter can play an important role working as a shelter for certain bacteria. In the river catchment, markers decreased 1 log after a rank of 200m to 2.6km depending the season and marker.

FEMS7-1193
Environmental Microbiology/Microbial Ecology /Microbial Communities

DYNAMICS OF MICROBIAL COMMUNITIES IN THE DECOMPOSITION OF LEAF LITTER IN FOREST ENVIRONMENTS

A. Bani[1], L. Borruso[1], S. Pioli[1], C. Wellstein[1], L. Brusetti[1]
[1]Free University of Bolzano, faculty of science and technology, Bolzano, Italy

Backgrounds

Litter decomposition is a key step in carbon flux cycle, in soil formation and stabilization. Fungi exert the major role in the decomposition and only recently has been given more attention to the bacterial community.

Objectives

This study aims to highlight the role of the microbial community involved in leaf decomposition in the mountain areas of South Tyrol (Italy). The goal is to study the decomposition of litter fall in their natural environment and in a transplantation experiment, simulating an environment with increased average temperature.

Methods

We selected litters characterized by tree different plant species and altitudes: oaks forest at 500, beech at 1000 and rhododendron at 1500 m.a.s.l. The decomposition was studied using *in situ* litterbags. In each sampling sites, litter bags containing leaves from own sites and from the others two sites were located and left for two years. The investigation foresaw the use of biomolecular fingerprinting techniques, Real Time PCR, Thermogravimetric analysis of the litter and enzymatic activity. In addition, environmental variables have been measured.

Conclusions

The results showed that the bacterial community is strongly affected by the sampling time, supporting the idea of a temporal succession of different taxa. Fungal community behaved differently with not clear assemblages. Within each single event, the plant species was the main driver of the bacterial community composition in all the time points, whereas fungal community showed a similar trend only in the first year of the experiment. Other environmental variables, such as average temperature, seemed to have little importance.

FEMS7-1869
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECT OF OZONE ADDITION TO CONTROL GORDONIA FOAMING ON THE NITRIFYING BACTERIAL COMMUNITIES IN A MUNICIPAL WASTEWATER TREATMENT PLANT

P. Barbarroja[1], *A. Zornoza*[1], *J.L. Alonso*[1], *S.V. Marin*[2]
[1]*Universitat Politècnica de València, Instituto de Ingenieria del Agua y Medio Ambiente, Valencia, Spain*
[2]*Universidad de Santander, Facultad de Ciencias Exactas- Físicas y Naturales, Bucaramanga, Colombia*

Backgrounds

The ozonation of activated sludge has been used as a technical measure for bulking control in a high number of full-scale wastewater treatment plants (WWTP), despite of the imprecise predictions on the level of reduction in filament grow or the lack of knowledge on impact on microbial community. Studies to elucidate the impact of this process on nitrifying bacterial community have not been carried out to date.

Objectives

The aim of this study was to evaluate the use of ozone for *Gordonia foaming* elimination on dynamic population of nitrifying bacterial community and nitrification performance of activated sludge system.

Methods

Full-scale studies were conducted over a period of 12 months at the two bioreactors of municipal WWTPs of Castellón (Spain). Both bioreactors were treated with different ozone dosages during this period. Samples from activated sludge, influent and treated effluent were collected every fifteen days and nitrifying community structures were analyzed using quantitative fluorescence in situ hybridization (qFISH). We investigated models of environmental interpretation of biological variables using of distance-based linear models (DISTLM).

Conclusions

Nitrosomonas oligotropha and members of the genus *Nitrotoga* were found by FISH as the dominant nitrifiers responsible for ammonia and nitrite oxidation, respectively. The DISTLM analysis showed that different ozone dosages determined the nitrifying activity, although ozone not appears to be an environmental factor influencing the dynamic of nitrifying bacterial community.

FEMS7-2373
Environmental Microbiology/Microbial Ecology /Microbial Communities

COMPARISON OF NITRIFYING MICROBIAL COMMUNITIES OF TWO FULL-SCALE MEMBRANE BIOREACTORS TREATING WASTEWATERS FROM MUNICIPAL SOLID WASTES USING 16S RDNA GENE AMPLICON SEQUENCING

P. Barbarroja[1], L. Moreno-Mesonero1[1], A. Zornoza[1], J. Fernández-Navarro1[1], J.L. Alonso[1], F. Muñagorri[2], C. García[2], C. Álvarez[2]
[1]Universitat Politècnica de València, Instituto Universitario de Ingeniería del Agua y Medio Ambiente, Valencia, Spain
[2]URBASER, Camino de las Hormigueras 171- 28031, Madrid, Spain

Backgrounds

Landfill leachate is a complex composition containing high levels of ammonia nitrogen. Membrane bioreactor systems (MBRs) are used for this type of complex wastewater treatment, because of their capacity of working at high concentrations levels of suspended solids and high cell retention times.

Objectives

In this study, 16S rRNA gene amplicon sequencing (NGS) combined with fluorescent *in situ* hybridization (FISH) were performed to identify detailed changes of nitrifying bacterial communities in activated sludge of two full-scale MBRs treating the waste effluents from landfill leachate treatment systems

Methods

Samples from activated sludge were collected monthly during fifteen months from two MBR systems. The nitrifying bacterial communities were analyzed using 16S rRNA gene amplicon sequencing (NGS) and fluorescence in situ hybridization (FISH). Distance-based linear models (DISTLM) were applied to investigate models of environmental interpretation of biological variables.

Conclusions

The two MBRs had different nitrifying microbial community compositions. In the MBR treating the waste effluents with high solids anaerobic digestion the ammonia oxidizing community was predominated by the family *Nitrosomonadaceae* y and the genus *Nitrosococcus*. In the MBR treating waste effluents with low solids the family Nitrosomonadaceae predominated but the genus *Nitrosococcus* was present a considerable lower abundances. We investigated models of environmental interpretation of nitrifying variables using of distance-based linear models (DISTLM).

FEMS7-1963
Environmental Microbiology/Microbial Ecology /Microbial Communities

EVIDENCES FOR A PHYSIOLOGICAL LINK BETWEEN HYDROCARBONS AND LIPIDS DEGRADATION IN THE MARINE ENVIRONMENT

C. Barnier[1], R. LAMI[2], P. LEBARON[2], L. INTERTAGLIA[3], F. HAKIL[1], P. SIVADON[1], L. URIOS[1], R. GRIMAUD[1]
[1]IPREM UMR CNRS 5254, EEM, Pau Cedex, France
[2]UPMC- Observatoire Océanologique de Banyuls-sur-Mer,
Laboratoire de Biodiversité et Biotechnologies Microbiennes, Banyuls-sur-Mer, France
[3]UPMC- Observatoire Océanologique de Banyuls-sur-Mer, CNRS- UMS 2348, Banyuls-sur-Mer, France

Backgrounds

Marine hydrocarbonoclastic bacteria belonging to the genera, among others, *Alcanivorax*, *Marinobacter*, *Cycloclasticus* and *Thalassolituus* have been recognized as main actors of the biodegradation of hydrocarbons in marine environment. Assimilation of these nearly water insoluble substrates involves the formation of a biofilm at the oil-water interface, which improves mass-transfer of hydrocarbons to the cells. Many of these strains have been described as having a very narrow substrate range mostly restricted to hydrocarbons. However, *Marinobacter hydrocarbonoclasticus* SP17 is able to assimilate a wide range of hydrophobic organic compounds (HOC) including alkanes and various lipids through the formation of an oleolytic biofilm at the HOC-water interface.

Objectives

Evaluate the diversity of oleolytic bacteria and their substrate range to determine if there is a link between hydrocarbon and lipid degradation.

Methods

A collection of more than 200 marine strains was screened for oleolytic biofilm formation on paraffin, triglycerides, fatty acids and wax esters.

Conclusions

About 25% of the tested strains were able to form a biofilm on at least one HOC, wax ester being the most utilized substrate. Most of the strains forming a biofilm on alkanes do so on triglycerides or/and wax esters suggesting that marine hydrocarbonoclastic bacteria are not restricted to the degradation of hydrocarbons. This also hints to the existence of a physiological link between the assimilation of hydrocarbons and lipids.

FEMS7-1186
Environmental Microbiology/Microbial Ecology /Microbial Communities

ONE YEAR SURVEY OF SOFT ROT ENTEROBACTERIA ALONG THE RIVER DURANCE ANTHROPOGENIC GRADIENT

M.A. barny[1], L. Blottiere[1], A. Lecomte[1], E. Rochelle Newall[1], F. Van Gijsegem[1], O. Berge[2]
[1]UPMC, IEES, PARIS, France
[2]INRA, Plant pathology, Monfavet, France

Backgrounds

Soft rot enterobacteria (SRE: Pectobacterium spp. and Dickeya spp.) infect a large number of plant species worldwide, including economically important plants. While the diversity of SRE observed on plant is fairly well described, the presence and diversity of SRE outside the plant context is not known in detail.

Objectives

To anticipate disease emergence from environmental reservoirs and to propose regulatory guidelines and good practices for crop health management it is important to gain knowledge of SRE ecology outside of agronomic context.

Methods

SRE sampling was performed all along the Durance river catchment in winter, spring, summer and autumn 2016. This river catchment is interesting because it linked alpine stream above the Serre-Ponçon lac to the Mediterranean agricultural basin of Avignon along an anthropogenic gradient. SRE sampling was coupled to detail analysis of water physicochemical characteristics. Diversity of isolated SRE was further characterized through sequencing of one house-keeping gene.

Conclusions

SRE repartition along the river stream was uneven, subjected to seasonal variation, land use and water physicochemical characteristics. This study further revealed a large previously unrecognized SRE diversity and pointed differential ecological behaviours of Pectobacterium spp. and Dickeya spp. The potential virulence of sampled SRE on various crops is currently under investigation.

FEMS7-1144
Environmental Microbiology/Microbial Ecology /Microbial Communities

ISOLATION AND CHARACTERIZATION OF B-TRIKETONE HERBICIDES DEGRADING BACTERIA ISOLATED FROM AN ARABLE SOIL

L. Barthelmebs[1], S. Romdhane[1], M. Devers-lamrani[2], C. Bertrand[3], F. Martin-laurent[2]
[1]University UPVD, BAE, Perpignan CEDEX, France
[2]INRA, Agroecology, Dijon, France
[3]University UPVD, CNRS, Perpignan CEDEX, France

Backgrounds

Microbial degradation is considered as the major process involved in the elimination of pesticides in the environment.

Objectives

In this context, our work aims to study the role of soil bacteria in the degradation of synthetic and natural β-triketone herbicides and to elucidate genetic mechanisms involved in their biotransformations.

Methods

Therefore, a microcosm approach was performed using an agricultural soil, in order to isolate bacterial strains capable of degrading β-triketone herbicides.

Conclusions

Two bacterial strains were isolated : *Bradyrhizobium* sp. SR1 able to degrade sulcotrione and mesotrione, two synthetic herbicides, and *Methylophilus* sp. LS1 degrading leptospermone, a natural one. The metabolism of these compounds was studied showing that their biodegradations resulted in the formation of several metabolites previously described in the literature, such as 2-chloro-4-mesylbenzoic acid, 4-methylsulfonyl-2-nitrobenzoic acid and 2-amino-4 methylsulfonylbenzoic acid from the two synthetic compounds, but also of two new metabolites identified as hydroxy-sulcotrione from sulcotrione and hydroxy-leptospermone from leptospermone.
In order to characterize degradation genes from *Bradyrhizobium* sp. SR1, a library of 14 000 mutants was constructed allowing the selection of two Sul^- mutants. The interrupted genes didn't code for sulcotrione degradation enzymes, but for transcriptional regulatory genes potentially involved in the regulation of stress. In the case of mesotrione, a nonspecific system involving a nitroreductase was identified. For leptospermone, the natural herbicide, degradation by *Methylophilus* sp. LS1 was most likely supported by a nonspecific system.
Our study reveals the existence of several degradation strategies used by soil bacteria for β-triketone herbicide degradation.

FEMS7-1535
Environmental Microbiology/Microbial Ecology /Microbial Communities

MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF PSEUDOMONAS SYRINGAE PV. ACTINIDIAE (PSA) FROM CHILEAN INFECTED KIWIFRUIT ORCHARDS

O. Flores[1], M. Nuñez[1], X. Besoain[2], C. Yañez[1], R. Bastías[1]
[1]Pontificia Universidad Católica de Valparaíso, Instituto de Biología, Valparaíso, Chile
[2]Pontificia Universidad Católica de Valparaíso, Escuela de Agronomía, Valparaíso, Chile

Backgrounds

In the last years the Chilean kiwifruit production has been affected by the phytopathogen *Pseudonomas syringae* pv. *actinidiae* (Psa). However few studies have been conducted about their phenotypic and genetic variability.

Objectives

The objetive of this work was to perform a molecular and phenotypic characterization of 18 Psa isolates obtained from infected Chilean kiwifruit orchards in 2012 and 2016.

Methods

The identity of Psa isolates was confirmed by conventional and duplex PCR with primers specific for Psa. Genetic variability of isolates was analyzed by Box/Eric-PCR fingerprinting and Multi Locus Sequence Typing (MLST) of four housekeeping genes sequences (*gyrB, rpoD, gltA* and *gapA*). For the phenotypic characterization, growth kinetic parameters, biofilm formation, phytohormone (AIA) production, and streptomycin/copper resistance were analyzed. The biochemical activity of isolates was determined by BIOLOG GEN III system.

Conclusions

MLST dendrogram analysis suggest that the Psa Chilean isolates are genetically homogenous and belong to biovar 3. Similar results were obtained by Box/Eric-PCR fingerprinting, which only one isolate presenting a slightly different pattern. However, we found differences in 21 of 94 biochemical assays. According to our results, all Chilean Psa isolates are able to produce the auxin IAA and present biofilm formation at 4°C. No isolate resistant to streptomycin or copper were detected. This work confirms the high genetic homogeneity between Chilean Psa isolates obtained from different geographic origin, which is in contrast with the phenotypic variation found in the biochemical activity. The biochemical characterization could be a good alternative to distinguish the different Psa isolates present in Chile.
FONDEF ID15I10032

FEMS7-0056
Environmental Microbiology/Microbial Ecology /Microbial Communities

INVESTIGATION OF VIABLE VIBRIO SPP. DYNAMIC IN NW MEDITERRANEAN COASTAL AREA USING A ONE STEP MULTIPLE OLIGONUCLEOTIDE FLUORESCENT PROBES AND SOLID PHASE CYTOMETER

J. Baudart[1], L. Girard[2], S. Peuchet[2], P. Servais[3], A. Henry[4], N. Charni-Ben-Tabassi[4]
[1]Sorbonne Universités- Université et Marie Curie Univ. Paris 06- CNRS, Observatoire Océanologique-Laboratoire de Biodiversité et Biotechnologies Microbiennes LBBM, Banyuls sur mer, France
[2]Sorbonne Universités- Université Pierre et Marie Curie- Univ. Paris 06- CNRS, Observatoire Océanologique-Laboratoire de Biodiversité et Biotechnologies Microbiennes LBBM, Banyuls sur mer, France
[3]Université Libre de Bruxelles, Ecologie des Systèmes Aquatiques, Bruxels, Belgium
[4]Veolia Environnement, Centre d'Analyses Environnementales, Saint Maurice, France

Backgrounds

Vibrios are physiological sensitive to changing environmental conditions and they are able to quickly adapt to these changes making them highly dynamic at short-term and seasonal scales.

Objectives

We investigated the weekly dynamic of viable vibrios cells in seawater over a 10-month period at two northwestern Mediterranean coastal sites through a high resolutive FISH-based method.

Methods

The Direct Viable Count assay was combined to a Fluorescent In Situ Hybridization approach using a one step-multiple oligonucleotide probes and the Solid Phase Cytometry (DVC-FISH-SPC) to monitor the total viable vibrios, in comparison to culturable counts. A multivariate analysis was used to identify environmental parameters explaining the abundances of culturable and total viable cells.

Conclusions

The specificity of the FISH approach was obtained by the combination of three different probes targeting specific sequences located at different positions on 16S rRNA of Vibrio and Aliivibrio members. Counts of total viable and culturable vibrios showed the same temporal pattern during the warmer season, but the ratios between both counts were inverted during the colder seasons (<16°C). We make the evidence that an important part of the viable vibrios are non-culturable during the colder seasons and that the seawater surface temperature identified as the main factor explaining the variations of culturable populations, was not an explanative variable for the total viable vibrios. The results highlighted the importance in the choice of the methodological approach to investigate the dynamic of viable vibrios in coastal water

FEMS7-1068
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE IMPACT OF INCREASED SANDSTORM ACTIVITIES ON THE RED SEA MICROBIOTA

H. Behzad[1], H. Ohyanagi[1], K. Mineta[1], Y. Saito[1], T. Gojobori[1]
[1]King Abdullah University of Science & Technology-KAUST,
Computational Bioscience Research Center CBRC / Biological and Environmental Sciences and Engineering BESE, Thuwal Jeddah, Saudi Arabia

Backgrounds

Large areas of lands around the world are faced with increased desertification and sandstorm activities, in part due to global warming effects. From the perspective of global environmental assessment, it is important to examine whether sandstorms can have significant impacts on microbial community structures in marine environments. We, thus, examined the effect of sandstorms on microbial communities in the Red Sea, since the Red Sea is a relatively isolated seawater region with no river inflow, minimal precipitation, and in close proximity to large deserts.

Objectives

To study the impact of increased dust activities on the Red Sea microbiota.

Methods

Metagenomics was used to investigate potential changes in microbial diversity in the Red Sea in response to two major sandstorms that severely impacted the Red Sea region in Sep 7-9 2015 and July 24-25 2016. DNA was extracted from the Red Sea water samples at different time points before and after sandstorms, 16S rRNA gene amplicon libraries were prepared and sequenced using Illumina MiSeq platform.

Conclusions

Bioinformatics analysis by QIIME revealed a consistent increase in the abundance of reads corresponding to Prochlorococcus, Archaea, and Bacteroidetes only in samples collected immediately following sandstorms. In addition, a number of Bacteroidetes phyla belonging to the order Flavobacteriales were present only in sandstorm-associated samples. Our results suggest that increased dust activities can change the relative abundance and composition of microbial communities in the Red Sea. We concluded that increased desertification and sandstorm activities due to global warming could lead to significant changes in marine environments.

FEMS7-1255
Environmental Microbiology/Microbial Ecology /Microbial Communities

BIOPESTICIDE ACTIVITY OF BACILLUS ATROPHAEUS STRAIN L193 AGAINST RHOPALOSIPHUM PADI

M. Rodríguez[1], A. Marín[1], M. Torres[1], I. Sampedro[1], L. Fernández[2], M. Campos[2], V. Béjar[1]
[1]Universidad de Granada, Microbiología, Granada, Spain
[2]Estación Experimental del Zaidín-CSIC, Protección Ambiental, Granada, Spain

Backgrounds

Rhopalosiphum padi is an aphid species that affects several cereal crops producing severe economic losses. Many depredator insects are used for its control in greenhouse crops; however the efficacy of these treatments in open fields is very low. Interestingly, within the genus *Bacillus* there are different species with insecticidal activity that could be used for the bio-control of *R. padi* due to the production of toxins, enzymes and surfactants.

Objectives

To determine the insecticidal activity of the *Bacillus atrophaeus* strain L193 against *R. padi*, and to characterize the compounds responsible for it.

Methods

The strain L193 was selected after a screening conducted with halotolerant strains in search of producers of surfactant and hydrolytic enzymes in order to find insecticidal activity. The biopesticide activity was tested using the purified surfactant, the culture supernatant and the whole culture by applying it on barley seedlings infested with *R. padi*. The type of surfactants was determined by mass spectrometry and by PCR using specific primers.

Conclusions

Strain L193 produces proteases, lipases, chitinases and glucanases. In addition, it produces 0.8 g/L of surfactants in TSB medium, which is a highly efficient compared to surfactants produced by other strains. The produced surfactants are surfactins and fengycins. The purified surfactants, the supernatant and also the whole culture produced a significantly higher mortality of *R. padi* than the control with a confidence level higher than 0.95. This strain could, therefore, be used for the biological control of aphids in open field crops.

FEMS7-2393
Environmental Microbiology/Microbial Ecology /Microbial Communities

PLANT-GROWTH PROMOTING PROPERTIES OF BACILLUS VELEZENSIS XT1, AN HALOTOLERANT BACTERIUM ISOLATED FROM A SALINE SOIL LOCATED IN SOUTH OF SPAIN

M. Torres[1], I. Llamas[1], E. Quesada[1], V. Béjar[1]
[1]University of Granada, Microbiology, GRANADA, Spain

Backgrounds

The plant-growth promoting rhizobacteria (PGPR) are microorganisms which live in soil near roots. Some of them control the pathogens of plants and moreover act as fertilizers. *Bacillus velezensis* XT1 is an halotolerant bacteria with PGPR properties.

Objectives

The aim of this work is to study the effect of *B. velezensis* strain XT1 in the growth of various horticultural species and the biochemical characteristics related to this activity.

Methods

The PGPR characteristics analyzed were nitrogen fixation, phosphate solubilization, and production of hydrolytic enzymes and siderophores. *In vivo* studies were performed in outdoor and in greenhouse crops. The statistical tests were done with *Statgraphics Centurion XVII* and *R* software.

Conclusions

The strain XT1 significantly increased the total weight (g/plant) and height (cm/plant) of tomato, pepper, squash and cucumber, when they were cultivated outdoors in pots during 50 days. The average values of the increase of these parameters were respectively (%): tomato, 52 and 31; pepper, 63 and 22; squash, 129 and 10; and cucumber, 101 and 8. Moreover, the number of fruits and flowers increased significantly in all the experiments. In addition, the crops of tomatoes in greenhouse, which were grown during 75 days, increased significantly the biomass area and the height (41 and 22% respectively). The metabolic activities of XT1 related to its PGPR properties were: the ability to fix nitrogen, solubilize organic and inorganic phosphate, produce siderophores, proteases, glucanases and ACC deaminase. The main conclusion is that the strain XT1 is a strong biostimulant of plant growth.

FEMS7-2487
Environmental Microbiology/Microbial Ecology /Microbial Communities

DOES SHEWANELLA LOHICA BIOREDUCE IRON OXIDES IN ANAEROBIC SEA SEDIMENTS?

R. Benaiges[1], J. Palau[2], E. Torres[2], J. Cama[2], J. Urmeneta[1,3], B. Dold[4,5]
[1]Universitat de Barcelona, Department of Genetics- Microbiology and Statistics, Barcelona, Spain
[2]IDAEA- CSIC, Institute of Environmental Assessment and Water Research, Barcelona, Spain
[3]Universitat de Barcelona, Biodiversity Research Institute IRBio, Barcelona, Spain
[4]Luleå University of Technology,
Department of Civil- Environmental and Natural Resources Engineering, Luleå, Sweden
[5]Sustainable Mining Research & Consultancy, EIRL, San Pedro de la Paz, Chile

Backgrounds

Shewanella is a well known genus of marine bacteria capable to perform the dissimilatory reduction of heavy metals. Previous studies have shown that *Shewanella loihica* is able to reduce not only soluble iron compounds but also magnetite in mineral form through polysaccharide attachment. However, reduction of natural iron oxides in marine conditions has not been studied yet.

Objectives

The main goal is the quantitative understanding of the capacity of *Shewanella loihica* to bioreduce natural iron oxides (e.g., magnetite) contained in marine sediments under anoxic conditions

Methods

In this study, natural iron oxides from different ore deposits were characterized and cultured along with *Shewanella loihica* strain PV-4 in microcosm batch experiments with a specific media that consists of synthetic sea water and lactate.

Conclusions

The results obtained showed an increase in dissolved iron concentration (up to 1000-50.000 µg/L) which suggests a significant *Shewanella*-mediated Fe reduction in all the studied iron oxides, in contrast to abiotic experiments, proving the existence of iron oxide bioreduction in anoxic marine sediments. The differences in dissolved iron concentrations from the microcosms prepared with different iron oxides are in part associated to their different specific surface area. Due to low iron bioavailability in the ocean, the bioreduction of mine oxide tailing disposed on the anoxic seafloor could have a major ecological impact. Moreover, this study may have implications for the assessment of the environmental impact of heavy metals contaminated sites as well as for potential biotechnology applications.

FEMS7-1466
Environmental Microbiology/Microbial Ecology /Microbial Communities

HARNESSING LONG READ SMRT SEQUENCING TECHNOLOGY FOR NATURAL PRODUCT GENE DISCOVERY IN POLAR DESERT SOILS

N. Benaud[1], E. Zhang[1], J.A. Kalaitzis[1], B.C. Ferrari[1]
[1]University of New South Wales, School of Biotechnology and Biomolecular Sciences, Sydney, Australia

Backgrounds

The majority of clinically useful antibiotics are derived from microbial natural products, typically produced through non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) pathways. Prolific biosynthetic Phyla include environmentally ubiquitous Actinobacteria, Proteobacteria, Firmicutes and Cyanobacteria.

Objectives

To combat the growing global antibiotic resistance crisis, novel chemical structures are urgently required. Extreme environments are expected to harbour unique microorganisms whose metabolic adaptations may also include novel natural products; however, this remains under-investigated, particularly as the majority of microorganisms defy cultivation. Next-generation sequencing offers the ability to screen environmental samples for NRPS and PKS genes.

Methods

Here, we have performed the first extensive survey of natural product-encoding genes in a range of Antarctic and High Arctic polar soils, where environmental conditions are some of the most extreme on earth, and where microorganisms dominate. Third generation PacBio ® SMRT sequencing offers longer reads than next-generation methods, as well as improved performance for GC-rich sequences. We will discuss the use of this platform to identify NRPS and Type I PKS gene sequence diversity from > 200 soil samples, spanning 12 polar sites.

Conclusions

Our findings reveal NRPS are more widely distributed in polar soil than Type 1 PKS genes. Additionally, recovered NRPS and PKS reads exhibited low amino acid sequence identity (%) with known biosynthesis proteins, indicating a high potential for novel compound production. We believe this workflow is highly relevant and applicable to a range of environmental samples, with the long reads provided by the SMRT platform delivering enhanced taxonomic resolution over second generation approaches.

FEMS7-1797
Environmental Microbiology/Microbial Ecology /Microbial Communities

VISUALIZATION OF MICROMONOSPORA IN NITROGEN FIXING NODULES

P. Benito[1], *M.E. Trujillo*[1]
[1]*University of Salamanca, Microbiology and Genetic, Salamanca, Spain*

Backgrounds

Nodular tissues can be considered an excellent habitat for bacteria, not only for nitrogen fixing rhizobia; the number of reports related to the presence of non-rhizobial microorganisms associated with nitrogen fixing nodules has increased in the past ten years. *Micromonospora*, a filamentous actinobacterium has also been reported as a normal inhabitant of actinochizal and legume nodules, but hitherto, the specific localization of the bacterium within the nodule structure or in relation to the nitrogen fixing bacteria is unknown. This information is key to understand their role in this plant-microbe interaction.

Objectives

The aim of this work was to locate *Micromonospora* in nodules of *Lupinus* plants coinoculated with *Bradyrhizobium* and to determine its occupancy within the different zones of a determinate nitrogen fixing nodule and the nitrogen fixing bacterium.

Methods

Lupinus albus plants were inoculated with *gfp*-tagged *Micromonospora lupini* (ML01) and the wild-type strain, *Bradyrhizobium* (CAR 08). After 40 days, mature nodules were examined using confocal laser scanning microscopy (CLSM), and transmission electron microscopy (TEM) coupled with immunogold labeling with antibodies raised against *gfp.*

Conclusions

Confocal microscopy showed that fresh effective lupine nodules contained *Micromonospora* expressing *gfp* and that it was located in the internal cortex and infected zones together with *Bradyrhizobium*. Moreover, immunogold labeling specifically localized *Micromonospora* within the infected plant cells. Taken together, our results demonstrate that the combined use of these two techniques provided a very strong evidence for infection of *Lupinus* by *Micromonospora* presenting clear evidence of the presence of this bacterium in nitrogen fixing nodules.

FEMS7-0720
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECT OF DIET AND ANTIBIOTIC TREATMENT ON THE HINDGUT MICROBIOTA OF RETICULITERMES GRASSEI, A LOWER TERMITE

M. Berlanga[1], *G. Ricardo*[2]
[1]*University of Barcelona, Biology- Health and Environment, Barcelona, Spain*
[2]*Academia europaea, Barcelona Knowledge Hub, Barcelona, Spain*

Backgrounds

Insects form a myriad of associations with microorganisms, ranging from pathogenic to intracellular symbiotic relationships. Lower termites are key models to understand insect-microbe symbiosis because of the diversity, complexity and functionality of their unique tripartite symbiosis (host, bacteria, and protists).

Objectives

Ecological interactions among members of the microbial communities may have differing net impacts on host fitness according to the actual environmental circumstances. We analyzed two aspects of the environment, nutrition and antibiotic, to show the shaping of the responses of the holobiont system.

Methods

We compared gut microbiota of *Reticulitermes grassei* lower termite using NGS to determine 16S rRNA microbial communities in control individuals (wood diet and no antibiotic treatment) versus individuals with a diet of cellulose and individuals with a diet of cellulose and antibiotic treatment (ciprofloxacin). All individuals were from the same termite colony.

Conclusions

Our results revealed that the hindgut prokaryotic communities in *Reticulitermes* (in all environmental conditions) are dominated by members of four Bacteria phyla: Bacteroidetes, Spirochaeta, Firmicutes, Proteobacteria, and Actinobacteria. Community similarity based on phylogenetic relatedness by unweighted UniFrac analyses indicated that the composition of the bacterial community in *Reticuliermes* with a wood or cellulose diet was no significantly different ($P \geq 0.05$), but it was significantly different in termites treated with ciprofloxacin versus termites without the treatment (wood or cellulose diet).

FEMS7-1750
Environmental Microbiology/Microbial Ecology /Microbial Communities

CRUDE OIL BIODEGRADATION BY MICROCOCCUS LUTEUS ATCC 4698

S. Bilen Ozyurek[1], *N.H. Avcıoglu*[1]
[1]*Hacettepe University Faculty Of Science, Biology/biotechnology, Ankara, Turkey*

Backgrounds

The leakage from oil and gas pipelines and oil wells, wrong methods for removing oil wastes, various human activities and accidental oil spills are among the main reasons for the pollution of environment by crude oil. Approximately 25% of oil contaminated areas are cleaned with natural remediation methods. In this context, various microorganisms play an important role in the environmental removal of oil pollutants.

Objectives

Determination of crude oil biodegradation with microbial growth intensities, gravimetric analysis and Gas Chromatography (GC) analysis by oil field isolated *Micrococcus luteus* ATCC 4698.

Methods

The microorganism was incubated under appropriate conditions in Bushnell Haas Broth medium containing oil as the sole carbon and energy source. After the incubation period, bacterial growth by utilizing crude oil was detected via measuring the turbidity of microbial suspension with UV-visible spectrophotometer at 600 nm against a blank. For the estimation of crude oil biodegradation by gravimetric analysis, residual crude oil was extracted with dichloromethane (DCM) (1:2). The crude oil biodegradation was calculated from the difference between initial and final concentrations of crude oil. Biodegradation ratios of hydrocarbons constituting the content of crude oil were determined by GC analysis.

Conclusions

As a result, the crude oil biodegradation efficiency of *Micrococcus luteus* ATCC 4698 was detected. In this regard, it was determined that this strain may be an alternative to advanced bioremediation studies.

FEMS7-1773
Environmental Microbiology/Microbial Ecology /Microbial Communities

INVESTIGATION OF THE DIFFERENT PHYSIOLOGICAL CONDITIONS ON PETROLEUM BIODEGRADATION BY RHODOTORULA MUCILAGINOSA

S. Bilen Ozyurek[1], N.H. Avcıoglu[1], I. Seyis Bilkay[1]
[1]Hacettepe University Faculty Of Science, Biology/biotechnology, Ankara, Turkey

Backgrounds

Petroleum production, drilling operations and improperly abandoned oil wells are the most important sources of oil contamination in soil, surface and ground waters. Petroleum leads to economic losses due to organic pollution in groundwater as well as decreasing of agricultural productivity on soil. In addition to the physical and chemical methods used to remove petroleum pollution, biological remediation is one of the most preferred methods.

Objectives

Determination of optimum physiological conditions for effective petroleum biodegradation by *Rhodotorula mucilaginosa.*

Methods

It was investigated that the effect of different pH, temperature, petroleum concentration (%) and incubation conditions on petroleum biodegradation by *Rhodotorula mucilaginosa.* To determine the petroleum biodegradation rates of bacteria, gravimetric analyses were carried out. The petroleum biodegradation rates were detected from the difference between initial and final concentrations of petroleum.

Conclusions

Petroleum biodegradation varies according to physiological conditions. In this manner, optimum physiological conditions for petroleum biodegradation are required to be determined for advanced bioremediation process.

FEMS7-3077
Environmental Microbiology/Microbial Ecology /Microbial Communities

RESPONSE OF ACTIVE BACTERIA TO ORGANIC AMENDMENT PRACTICES AND CONSEQUENCES ON VOLATILE ORGANIC COMPOUND (VOC) EMISSIONS FROM AGRICULTURAL SOILS

C. Monard[1], K. Potard[1], N. Le Bris[1], J.P. Caudal[1], J.L. Le Garrec[2], F. Binet[1]
[1]CNRS - University of Rennes 1, ECOBIO, Rennes, France
[2]CNRS-Université de Rennes 1, Institut de Physique de Rennes, Rennes, France

Backgrounds

Microbial degradation of soil organic matter (SOM) generates volatile organic compounds (VOCs) which may constitute a significant loss of soil C and take part in greenhouse gases production in the atmosphere. Since agricultural land uses may control the SOM decomposition by changing microbial communities and SOM sources, there is a need to assess the consequences of perennial organic waste inputs onto the soil on VOC emissions.

Objectives

Based on an experimental site dedicated to study the agronomic and environmental effects of livestock effluent inputs (EFELE, France), our study aimed to determine the response of soil living bacteria to organic amendments and the following VOC emissions by soil.

Methods

Just before and up to two months after the amendments, we simultaneously sampled soil and gases on plots receiving Pig Slurry (PS) or Methanised Pig Slurry (MPS) and Control (C) plots. The living bacterial diversity was analysed by Illumina sequencing and linked to the VOC emissions measured with a PTR-MS.

Conclusions

In all soils, the living bacterial communities were dominated by *Proteobacteria* and *Acidobacteria* and varied temporally. A 2-ways ANOVA of the VOC dataset indicated that seasonal pattern and organic manure inputs significantly changed the VOC emissions, manure effects being timely dependent. These spectra were dominated by methanol and acetonitrile, methanol emissions being stimulated after PS inputs leading to an increase in C-VOC fluxes from soil to the atmosphere. We showed that i) VOCs emitted by organic amended soils were not negligible, ii) soil organic amendments induced bacterial changes and iii) MPS inputs reduced VOC emissions by soils compared to PS ones which is of great interest to mitigate GHG emissions in agriculture.

FEMS7-0196
Environmental Microbiology/Microbial Ecology /Microbial Communities

NOVEL MICELLE PCR-BASED METHOD FOR ACCURATE, SENSITIVE AND QUANTITATIVE MICROBIOTA PROFILING

S. Boers[1], J. Hays[1], R. Jansen[2]
[1]Erasmus University Medical Centre, Department of Medical Microbiology and Infectious diseases, Rotterdam, The Netherlands
[2]Regional Laboratory of Public Health, Department of Molecular Biology, Haarlem, The Netherlands

Backgrounds

16S rRNA gene profiling has revolutionized the field of microbial ecology. Unfortunately however, microbiota profiling methods are semi-quantitative methods, where the microbiota results are presented as proportional abundances rather than absolute abundances. This restriction lowers the reproducibility of microbiota results and complicates cross-study comparability. Additionally, the validity of microbiota results is threatened by the presence of contaminating bacterial DNA derived from the laboratory environment and the reagents/consumables used.

Objectives

Development of a novel approach employing micelle PCR (micPCR) in combination with an internal calibrator (IC) that allows for standardization of microbiota profiles via their absolute abundances and subtraction of contaminating bacterial DNA.

Methods

MicPCR is a single-molecule clonal amplification method in which template DNA molecules are separated into a large number of physically distinct reaction compartments using a water in oil emulsion. This compartmentalization allows accurate quantification of target sequences due to a lower susceptibility to variations in PCR amplicon efficiencies and amplification biases. The addition of an IC allows to express the resulting operational taxonomic units (OTUs) as a measure of 16S rRNA gene copies. Further, the quantification of contaminating bacterial DNA via the use of negative extraction controls allows the subtraction of contaminant bacterial DNA from actual samples.

Conclusions

The micPCR/NGS approach generates accurate quantitative microbiota profiles that are free of contaminating bacterial DNA from environmental sources. The general adoption of this approach will greatly improve the standardization of microbiota profiling results between individual experiments, laboratories and scientific publications.

FEMS7-2124
Environmental Microbiology/Microbial Ecology /Microbial Communities

IN SILICO DETECTION OF HYDROLASES IN THE METAGENOME OF THE COLOMBIAN ANDEAN FOREST SOIL AND THEIR INDUSTRIAL IMPORTANCE

I. Bolaños[1], P. Velez[1], P. Moreno[2]
[1]Universidad del Cauca, Biologia, Popayan, Colombia
[2]Universidad del Valle, EISC, Cali, Colombia

Backgrounds

Colombia has an immeasurable environmental richness due to the variety of the environmental conditions given by a complex topography. For example, the Colombian Andean forest is home of incalculable species, such as the extremophile microorganisms from which the extremophilic enzymes (extremozymes) as esterases, Laccases, and hydrolases are obtained. Because their unique features, the hydrolases represent one of the most important enzymes of the pharmaceutical industry. The Colombian metagenome soil high Andean forest (BAA), located at the national natural park “Los Nevados” shows extreme environmental conditions in respect of the height, 3.590 masl, low temperatures, low oxygen levels and high radiation levels which makes it part of a very interesting reservoir of extremozymes. detection of hydrolases in the metagenome of the BAA was made by using the reads kept in the servers of the Colombian Center for Genomics and Bioinformatics of Extreme Environments (GeBiX).

Objectives

For that reason, the current investigation was focused on the detection of hydrolases deriving of the extremophile microorganisms from the BAA metagenome.

Methods

The detection of hydrolases in the metagenome of the BAA was made by using the reads kept in the servers of the Colombian Center for Genomics and Bioinformatics of Extreme Environments (GeBiX). The functional annotation was carried out through a BlastX analysis againts not redundant database of proteins of the NCBI and Pfam web sites.

Conclusions

This is one of the two main investigations of the high Andean forest metagenome allows to find that about 20% of the detected enzymes corresponds to the so-called hydrolase type. This finding, this is a base line of great interest for future biotechnology development from creation and evaluation of BAA metagenome libraries, with potential impact for studying the Colombian soils.

FEMS7-1760
Environmental Microbiology/Microbial Ecology /Microbial Communities

EXPLORING THE RHIZOSPHERIC MICROBIOME OF VITIS VINIFERA CV. PINOT NOIR USING METAGENOMIC AND METAPROTEOMIC APPROACHES: PHYLOGENETIC AND FUNCTIONAL CHARACTERIZATION

E. Bona[1], P. Cesaro[2], G. Novello[2], L. Boatti[3], V. Magnelli[2], N. Massa[2], M. Manfredi[4], G. Lingua[1], F. Mignone[2], E. Marengo[2], G. Berta[2], E. Gamalero[2]
[1]Università del Piemonte Orientale, Dipartimento di Scienze e Innovazione Tecnologica, Vercelli, Italy
[2]Università del Piemonte Orientale, Dipartimento di Scienze e Innovazione Tecnologica, Alessandria, Italy
[3]SmartSeq s.r.l., spin-off of the Università del Piemonte Orientale, Alessandria, Italy
[4]Isalit srl, spin-off of the Università del Piemonte Orientale, Novara, Italy

Backgrounds

Vitis vinifera is a Mediterranean crop with relevant impact on the Italian landscape, culture and economy. The rhizosphere is a hotspot where the release of root exudates stimulates bacterial density and diversity. Thanks to culture-independent methods (metagenomics) the complexity of the soil/rhizosphere microbial community has been explored. However, metagenomics doesn't provide information regarding the activity and the molecular interactions between the bacterial communities and roots. Proteins are the drivers of cellular activities encoded by the genome. Therefore, proteomic tools could be useful to gain information about microbial community activity and to better understand the real interactions pathways between roots and soil.

Objectives

Actinobacteria were the dominant class in all the soil samples, followed by Proteobacteria, Gemmatimonadetes and Bacteroidetes. While Actinobacteria and Proteobacteria are well known as dominant in soil, for the first time members belonging to Gemmatimonadetes have been observed in vineyard soils. Bacteria belonging to Streptomyces, Bacillus and Pseudomonas genera were the most active. The identified genera were mainly involved in phosphorus and nitrogen soil metabolism.

Methods

A comparison between the microbial community structure in rhizospheric and bulk soil using metagenomics (pyrosequencing of 16S rDNA) and proteomics (MS/MS analysis of the total protein occurring in soil samples) was performed.

Conclusions

Our results underlined the difference between the metagenomic and metaproteomic approaches and the potentiality of proteomics in describing the environmental bacterial communities and their activity.

FEMS7-2263
Environmental Microbiology/Microbial Ecology /Microbial Communities

TALES FROM THE UNDERGROUND: SPATIAL VARIATIONS IN THE ABUNDANCE OF ANTIBIOTIC RESISTANCE GENES AND PUTATIVE PATHOGENS IN SEWER BIOFILMS

C. Borrego[1], O. Auguet[2], M. Pijuan[2], X. Triadó-Margarit[3], S. Varela Della Giustina[4], S. Rodríguez-Mozaz[4], O. Gutiérrez[2]
[1]Catalan Institute for Water Research, Quality and Microbial Diversity, Girona, Spain
[2]Catalan Institute for Water Research, Technology and Evaluation, Girona, Spain
[3]Centre d'Estudis Avançats de Blanes CEAB-CSIC, Integrative Freshwater Ecology Group, Blanes, Spain
[4]Catalan Institute for Water Research, Quality, Girona, Spain

Backgrounds

A large part of antibiotics consumed by humans are not metabolised but excreted with urine and faeces and transported to wastewater treatment plants via sewer systems. Transport of wastewater along sewers promotes the development of biofilms, which are characterised by a high cell density and a heterogeneous composition. Sewer biofilms provide an optimal environment for the accumulation of antibiotic resistance genes (ARGs).

Objectives

Our main goal was to characterise the changes in both the abundance of ARGs and the composition of bacterial communities, including potential pathogens, in different sewer compartments (flowing wastewater and biofilms) along a sewer pipe (inlet/outlet).

Methods

The concentration of antibiotics and ARGs were analysed by UPLC-MS and qPCR, respectively, in biofilm and wastewater samples at the inlet and the outlet sections of a full-scale sewer. Composition of bacterial communities was assessed by high-throughput sequencing. Occurrence of potential pathogens was estimated by comparing representative OTU sequences against an in-house database of bacterial pathogens.

Conclusions

The abundance of antibiotic residues and ARGs was uncoupled in both sewer compartments (wastewater/biofilms). The relative concentration of *qnrS*, *sul1*, *sul2*, bla_{TEM} and *intI1* was significantly higher in biofilms at the inlet section of the pipe, whereas no spatial differences were observed neither for *ermB* in biofilms nor for any gene in wastewater. This spatial heterogeneity was congruent with differences in the composition of biofilm communities between inlet and outlet sections of the sewer, especially regarding the relative abundance of several well-known human pathogens that were significantly higher in inlet biofilms.

FEMS7-2783
Environmental Microbiology/Microbial Ecology /Microbial Communities

INCREASED NITROGEN DEPOSITION ON TREE LEAVES PHYLLOSPHERE AFFECTS FUNGAL COMMUNITY DIVERSITY BUT NOT BACTERIAL COMMUNITY

L. Borruso[1], A. Bani[1], M. Ventura[1], P. Panzacchi[1], L. Antonelli[2], G. Tonon[1], L. Brusetti[1]
[1]Free University of Bolzano, faculty of science and technology, Bolzano, Italy
[2]AIT Austrian Institute of Technology, Department of Health & Environment, Vienna, Austria

Backgrounds

In the last decades, atmospheric nitrogen deposition (N) onto forests has constantly increased and is expected to double by 2050. The increased N flux is likely to affect forest ecosystems, most of which are considered particularly sensible to increased N availability. Several studies have already investigated the effects of soil N fertilization on forests, evidencing negative effects on plant biodiversity and the associated biota. However, there is a lack of knowledge regarding the effects of N depositions applied directly on the tree canopies on the associated microbiota.

Objectives

This study aims to investigate the influence of increased N deposition on the microbial communities associated with leaves phyllosphere, in a temperate forest dominated by sessile oak (*Quercus petraea* Liebl.).

Methods

The study area is located in Monticolo natural reserve (South Tyrol, Italy) where a long-term ecosystem experiment has been established simulating increased N deposition. The experimental design consisted in three control plots and three plots treated with canopy N fertilization. A total of 20 kg N ha^{-1} y^{-1} were provided as NH_4NO_3, with five applications from May 2015 until September 2015. Fifteen days after the last fertilization, tree biological replicates were collected in each plot. Bacterial and fungal communities were investigated via Illumina Hiseq and quantitative PCR. N leaves concentration was measured via mass spectrometry.

Conclusions

Results highlight that increasing N deposition affect the fungal community diversity, even in the short term, whereas the bacterial community is not affected by the treatments.

FEMS7-3115
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE EFFECT OF GLOBAL WARMING ON COLD ADAPTED YEAST AND FILAMENTOUS FUNGI IN MIAGE GLACIER (ITALY)

C. Sannino[1], L. Borruso[2], S. Filippucci[1], G. Tasselli[1], P. Buzzini[1], L. Brusetti[2], B. Turchetti[1]
[1]University of Perugia,
Department of Agricultural Food and Environmental Science & Industrial Yeasts Collection DBVPG, Perugia, Italy
[2]University of Bolzano, Department of Science and Technology, Bolzano, Italy

Backgrounds

Cold environments have been considered for a long time habitat supporting only latent forms of microbial life. However, several studies have reported the presence of psychrophilic and psychrotolerant microorganisms, including fungi, in cold habitats. The first studies on cold adapted fungi were performed on Polar areas and only at the beginning of XXI century, where few authors reported the presence of eukaryotic microorganisms in Alpine glaciers.
Although Alpine glaciers represent an interesting habitat for microbial life, global warming is causing the dramatic retreat of ice-covered areas including glaciers, thus implying the potential loss of cold-adapted microbial biodiversity.

Objectives

The aim of the present study was to investigate the non-cultivable eukaryotic diversity (yeasts and filamentous fungi) of debris covering ice surface of Miage glacier (Italian side of Mont Blanc).

Methods

Replicates of debris samples from Miage glacier were collected in three consecutive years. A Next Generation Sequencing (NGS) approach (Illumina Miseq) has been used to amplify the ITS rRNA region. Sequences were used to study the non-cultivable yeast and fungal microbioma.

Conclusions

The biodiversity achieved by NGS may be an useful approach to investigate the diversity of yeast and filamentous fungi colonizing cold ecosystems of Alpine glaciers. The possible impact(s) of environmental changes on cold-adapted eukaryotic diversity is discussed

FEMS7-2096
Environmental Microbiology/Microbial Ecology /Microbial Communities

ASSESSMENT OF BACTERIAL DIVERSITY IN THE MOLNAR JANOS HYPOGENIC CAVE (HUNGARY)

A. Borsodi[1], D. Anda[1], A. Szabó[1], L. Jurecska[1], G. Krett[1], J. Mádl-Szőnyi[2], K. Márialigeti[1], J. Makk[1]
[1]Eotvos Lorand University, Department of Microbiology, Budapest, Hungary
[2]Eotvos Lorand University, Department of Physical and Applied Geology, Budapest, Hungary

Backgrounds

The Molnár János cave belonging to the northern Rose Hill discharge area of Buda Thermal Karst System (Hungary) is the largest active thermal water cave in Europe. Discharges of two types of water are characteristic for this area with different temperatures, therefore, mixed corrosion proved to be the dominant cave forming process. This study looked at different ecological niches (hot, cold and mixed water, sediment and reddish-brown biofilm on the subaqueous cave wall) within the phreatic zone of the cave system.

Objectives

Our study aimed to obtain a detailed view about the taxonomic diversity of microbial communities using next generation sequencing (NGS).

Methods

The samples were collected in January 2014. For the identification of bacteria primers specific for the V3-V4 region of the 16S rRNA gene were used and amplicons were analyzed on a Roche GS Junior platform.

Conclusions

Proteobacteria, Acidobacteria, Chlorobi, Chloroflexi and Nitrospirae were abundant in all samples. Dominance of ammonia-oxidizing bacteria (*Nitrosococcus*) and nitrite-oxidizing bacteria (*Nitropspirae*) were revealed from the cold water sample. Members of the phylum Nitrospirae were also abundant in the cave sediment sample. The mixed and hot water samples were predominated by the genus *Sulfuricurvum* related sequences belonging to the class Epsilonproteobacteria, but this class was almost completely absent in other samples. It seems that sulfide, oxygen and water flow characteristics were critical for niche differentiation among major bacterial groups participating in the sulfur cycle of Molnár János cave.

This research was supported by the Hungarian Scientific Research Fund (OTKA) Grant NK101356.

FEMS7-0061
Environmental Microbiology/Microbial Ecology /Microbial Communities

CAMPANOPHYLLUM: BIO-PROFILING THE ENDANGERED MUSHROOM FROM INDIA

M. Borthakur[1], *S.R. Joshi*[1]
[1]*North Eastern Hill University, Biotechnology and Bioinformatics, Shillong, India*

Backgrounds

Meghalaya, India located in the domain of mega-biodiversity hotspots, is an abode to diverse wild mushrooms.The present study dealt with exploration of a rare gilled Agaricales listed as endangered under IUCN A4c criteria due to its habitat decline.

Objectives

The current investigation was undertaken to assess the various nutritional, toxicological, antioxidant and bio-profiling of an endangered mushroom *Campanophyllum proboscideum* from a high altitude geographical area of Northeastern India.

Methods

The species was identified based on phenotypic and molecular characterization. Nutritional profiling was performed according to AOAC 1995. Phytoconstituents were characterized and Meixner test was performed. Bioactive components were determined including DPPH radical scavenging and antimicrobial efficiency evaluated against clinical pathogens. Gas Chromatographic profiling was performed to infer the active components as a natural dietary supplement.

Conclusions

The rare species was characterized as *Campanophyllum proboscideum* based on Neighbour joining (NJ) and Bayesian phylogeny. Being negative in Meixner, it doesnot contain hallucinic alkaloid. Moisture content was high with 76.58%, 5.6% of fiber and ash respectively in dried mass with protein content of 11.50±0.18.The extract exhibited the potent radical-scavenging activity at a maximum concentration of 10 mg/ml and the scavenging effect was 50.62%with IC_{50} at 94.46.The extract showed significant ($p < 0.05$) reducing power (1.285) and flavonoid content (0.94±0.037). β-carotene and lycopenes were 0.143±0.26µg/g and 0.141±0.14 µg/g respectively. The methanolic extract showed two-fold increase in antimicrobial activity when combined with common antibiotic Chloramphenicol. Bioactivity profiling revealed the presence of aliphatic hydrocarbons, terpene, alcohol, volatile organic compound and a phenolic compound, Phenol, 2, 4-Bis (1, 1-Dimethylethyl) (2, 4-DTBP).

This study indicates the endangered *Campanophyllum proboscideum* as potent source of supplementary food rich in natural antioxidants which can also be explored as a pharmaceutical agent.

FEMS7-1862
Environmental Microbiology/Microbial Ecology /Microbial Communities

EVALUATION OF CARBON AND NITROGEN SOURCES IN THE PRODUCTION OF BIOSURFACTANT BY A BACILLUS AQUIMARIS HALOPHILIC STRAIN ISOLATED FROM OIL RESERVOIR

K.F. Carvalho[1], M.N. Argentin[2], L.F. Martins[3], N.R.S. Bossolan[4]
[1]Federal University of São Carlos - UFSCar,
Graduate Program in Evolutionary Genetics and Molecular Biology, São Carlos, Brazil
[2]São Carlos Institute of Physics- University of São Paulo,
Graduate Program in Physics - Applied/Biomolecular option, São Carlos, Brazil
[3]PETROBRAS, CENPES - Petrobras Research & Development Center, Rio de Janeiro, Brazil
[4]São Carlos Institute of Physics- University of São Paulo,
Department of Physics and Interdisciplinary Science, São Carlos, Brazil

Backgrounds

Biosurfactants (BS) have potential to be applied in petroleum industry, as the case of advanced oil recovery processes using microorganisms and/or their products (MEOR). Production of BS is influenced and regulated by several factors, including carbon (C) and nitrogen (N) sources available, as well as the C/N ratio, therefore, the control of these factors is important to improve BS production.

Objectives

To evaluate the production of BS by *Bacillus aquimaris* O9-01 isolated from an oil reservoir, using different N and C sources and varying the C/N ratio, aiming to improve its BS production.

Methods

Bacillus aquimaris O9-01 was isolated from aerobic culture in LB broth (70 g/L salinity), at 37°C, inoculated with oil (10% v/v) from an offshore Brazilian oil reservoir. Mineral medium was used to test the N and C sources, as well C/N ratios, by varying one factor and keeping the other two fixed. The N sources tested were $NaNO_3$, NH_4Cl and NH_4NO_3 at a fixed concentration of 1.0 g/L; C sources were glycerol, glucose, mannose, kerosene, petrolatum and crude oil; and the C/N ratios 2, 4, 8, 16 and 20 (w/w). Bacterial growth was measured by optical density and BS production by emulsification index E24.

Conclusions

Glycerol, NH_4Cl and C/N 2 stimulated the largest BS production, reaching 67% E24 with 102 hours of incubation. These results indicate the potential for BS production by *B.aquimaris* O9-1 in a low C/N ratio and with a low cost carbon source.

FEMS7-0735
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE UNEXPLORED FUNGAL COMMUNITY OF THE SPONGE GRANTIA COMPRESSA: IDENTIFICATION AND METABOLIC FINGERPRINT

E. Bovio[1], L. Garzoli[1], D. Firsova[2], G.P. Mc Cormack[3], M. Mehiri[4], G.C. Varese[1]
[1]University of Turin, Department of Life Sciences and Systems Biology, Turin, Italy
[2]University of Ireland Galway, Marine Biodiscovery laboratory, Galway, Ireland
[3]University of Ireland Galway, Molecular Evolution & Systematics Laboratory, Galway, Ireland
[4]University Nice Sophia Antipolis, Institute of Chemistry of Nice, Nice, France

Backgrounds

Marine environment represents an untapped source of microbial diversity. Less than 0.1% of marine microorganisms are known, and only recently researchers have focused on marine fungi. These organisms are considered a valuable source of natural products, by the activation of different metabolic pathways. The One Strain-Many Compounds approach (OSMAC) is a powerful method to obtain these interesting secondary metabolites.

Objectives

The aims of this study were: i) to identify the fungal community associated with the sponge *Grantia compressa* to deepen the knowledge on sponge mycobiota; ii) to apply the OSMAC approach to highlight the metabolic potential of marine fungi in a biotechnological perspectives.

Methods

The mycobiota associated with *G. compressa* was isolated using different growth media and incubation temperatures, to increase the number of cultivable fungi. Fungal strains were identified combining morpho-physiological and molecular features. The OSMAC approach was applied to stimulate the production of metabolites of the isolated fungi, using 12 growth conditions, including a co-culture with marine bacteria. The crude extracts of fungi, grown in different conditions, were analyzed by HPLC-DAD to highlight each fungal metabolomic fingerprint.

Conclusions

The culturable mycobiota (18 taxa) of *G. compressa* is dominated by Ascomycota (72%), followed by Basidiomycota (28%). The preliminary chemical results showed that the OSMAC approach is a powerful technique to detect a high number of metabolites and to stimulate the expression of different metabolic pathways. The results of the chemical fingerprints lead to the selection of the best growth conditions for the isolation and identification of novel molecules for biotechnological applications.

FEMS7-1515
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE MICROBIOME IN HEALTH AND DISEASE: USING A FLY MODEL TO UNDERSTAND HOST-MICROBE INTERACTIONS

N. Broderick[1]
[1]*University of Connecticut, Molecular and Cell Biology, Storrs, USA*

Backgrounds

Gut microbiota provide key contributions to host health and many studies have correlated perturbations of the microbiota with disease. There is increasing data that the microbiome is a critical factor in the susceptibility and progression of disease. The *D. melanogaster*-microbiome system provides a simple model ecosystem to interrogate mechanisms of host disease ecology that are largely inaccessible in other systems.

Objectives

Here, we use two well characterized bacterial pathogens of *D. melanogaster*, *Pectobacterium carotovora* (*Ecc15)* and *Pseudomonas entomophila*, to explore the contribution of gut microbiota to host susceptibility.

Methods

We reared flies in the presence (conventional) or absence (axenic) of their microbiome and compared adult survival following infection with either *P. carotovora* or *P. entomophila*. Host survival, immune and stress responses, as well as microbiome and pathogen colonization were measured over time.

Conclusions

Flies reared without gut microbiota are less susceptible to oral infection by *P. entomophila and* exhibit lower immune and stress responses. Ingestion of *P. carotovora* is not lethal to wild-type flies, but injection into the fly hemocoel increases mortality. Initially, *P. carotovora*-infected axenic male flies die faster than conventionally reared flies. However, both male and female axenic flies show greater long-term survival than flies reared with microbiota. These results suggest *Drosophila* microbiota influence susceptibility to infection through interactions with the host immune and stress response, but that this effect can be dependent on sex, the route of infection, and assay duration.

FEMS7-2060
Environmental Microbiology/Microbial Ecology /Microbial Communities

DRINKING WATER MICROBIOME: A NEGLECTED BIODIVERSITY REVEALED

A. Bruno[1], A. Sandionigi[1], M. Bernasconi[2], E. Siani[1], M. Labra[1], M. Casiraghi[1]
[1]University of Milano-Bicocca, Biotechnologies and Biosciences, Milan, Italy
[2]Metropolitana Milanese spa, Metropolitana Milanese spa, Milan, Italy

Backgrounds

Drinking water shows a wide diversity of microorganisms interacting in a dynamic network. Recent studies revealed that drinking water treatment process can affect the microbiome community structure. Moreover, scientists recently reported the presence of neglected and poorly known biodiversity in groundwater.

Objectives

Drinking Water Treatment Plants (DWTPs) can be a source of unexpected biodiversity in terms of environmental microorganisms, still poorly known. Molecular techniques can give a deeper knowledge, going beyond the limit of culture-dependent methods.

We evaluated and standardized a new experimental workflow for microorganisms concentration, DNA extraction and amplification, suitable for molecular analysis and optimized for High-Throughput DNA Sequencing approaches.

Methods

A one-year sampling campaign was carried out in a DWTP in Milan (Italy) in collaboration with Metropolitana Milanese S.p.A. We collected samples at different steps of the potabilization processes, from groundwater, through carbon filters, to the last treatment (chlorination). Drinking water microbiome was investigated with Illumina MiSeq paired-end sequencing of 16S rRNA gene.

Conclusions

The results obtained showed the occurrence of unexplored bacteria in the entire DWTP, from groundwater, across carbon filters, to post-chlorination.

Secondly, our data suggested that carbon filters can be a substrate for microorganism growth and can contribute to seed water downstream, since chlorination does not greatly modify the composition of the incoming bacterial community.

Poorly known bacteria can be pivotal in the microbial dynamics of drinking water: DWTPs are complex ecosystems, where a neglected network of microbial interactions take place from the source to the tap in our house, raising new questions about drinking water management.

FEMS7-0125
Environmental Microbiology/Microbial Ecology /Microbial Communities

DETECTION OF THE ARXA GENE ENCODING ARSENITE OXIDASE IN VARIOUS ECOSYSTEMS BY USING NEWLY DESIGNED PCR PRIMERS

M.C. Cabrera Ospino[1], *H. Kojima*[1], *T. Watanabe*[1], *T. Iwata*[2], *F. Manabu*[1]
[1]*Hokkaido University, The Institute of Low Temperature Science, Sapporo, Japan*
[2]*University of Yamanashi, Environmental Sciences, Kofu, Japan*

Backgrounds

Arsenic (As) is a toxic metalloid found as inorganic arsenate (AsV) and arsenite (AsIII) in the environment. Some bacteria can use AsIII as an electron donor, utilizing one of the two arsenite oxidase, coded by *aio*, and *arx* genes. The latter has been found only in relatively limited number of organisms, and its diversity and distribution in the environment are hardly known. In a previous study, a primer pair to detect the *arxA* gene was designed from the sequences of gammaproteobacterial strains. With the primer pair, some *arx* gene sequences were directly detected in alkaline/saline environments. However, this primer pair failed to get a PCR product from arsenic rich sites with different pH. This may be related to the coverage of the primers, because the primers have many mismatches with the *arx* gene found in a betaproteobacterial strain.

Objectives

The aim of this study was to design a new PCR primer set in order to detect more diverse *arx*A sequences in various ecosystems.

Methods

The primer pair was designed on the basis of 20 *arx* gene sequences. It was experimentally confirmed that it can amplify the *arxA* gene from more diverse organisms. By using the new primer pair, diversity of *arxA* gene in some circumneutral sites was analyzed.

Conclusions

The partial *arxA* sequences obtained in this study were phylogenetically distinct from those of higher pH sites and the genes of the alkaliphilic isolates previously reported. This study provides an important proof of a wider diversity and distribution of *arxA* sequences in the environment.

FEMS7-2219
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROORGANISM ROLE ON CULTURAL HERITAGE LIFE

L. Scrano[1], S. Sasso[2], M.G. Bonomo[2], L. Calabrone[2], G. Salzano[2], R. Laviano[3], S.A. Bufo[2]
[1]Università degli Studi della Basilicata,
European and Mediterranean Cultures. Architecture- Environment and Cultural Heritage, Matera, Italy
[2]Università degli Studi della Basilicata, Sciences, Potenza, Italy
[3]Università di Bari, Earth and geo-environmental sciences, Bari, Italy

Backgrounds

Microorganisms play an important role in biodeterioration processes of cultural heritage, because they mostly represent the founder organisms of the ecological successions which develop on the artefact. The consideration of the artistic heritage as a sensing receptor, is an extremely complex issue: each material with morphological, chemical and physical properties totally dissimilar, makes it an "*unicum*", characterized by a specific vulnerability. Conservation of historic buildings and monuments is a major problem in modern societies from both an economical and cultural point of view. Solutions for the safeguard of cultural heritage are usually based on different procedures to remove microorganisms not taking into account that the 'Nature' creates an important balance between all living organisms. In fact, it is true that the bacteria create a substrate for successive specialized colonizers but it is also true that, on the stones, there are bacteria repairers of environmental damage*.*

Objectives

The aim of this research was to identifying and studying the role of microorganisms present on the stones of two historical bridges located in Basilicata region.

Methods

Identification of microorganisms collected from the bridges; evaluation of bacteria' role; assessment of the quality of the locally produced bio-calcite for the stone consolidation.

Conclusions

Microbial species identified on both the artifacts belong to Firmicutes, Actinobacteria and Proteobacteria phyla. Among the bio-consolidant bacteria, *Bacillus thuringiensis* has been demonstrated to produce the greatest amount of crystals, coherent with the limestone substrate.
"*We follow the nature, excellent guide as a deity and obey it*" (CICERO).

FEMS7-2480
Environmental Microbiology/Microbial Ecology /Microbial Communities

FOOD COMPONENTS AFFECT THE IN VITRO ADHESION OF ORAL BACTERIA TO THE INTESTINAL MUCOSAL NICHE

M. Calatayud[1], E. Hernandez-Sanabria[1], A. Rizky Ferdina[1], N. Boon[1], T. Van de Wiele[1]
[1]Ghent University, Center for Microbial Ecology and Technology CMET, Gent, Belgium

Backgrounds

The oral cavity is a dynamic environment with relevant impact on host physiology. Food and saliva are the main sources of bacteria incoming the small intestine, which potentially impact the structure and function of native intestinal microbial communities.

Objectives

Assessing the impact of food components (oil, meat, and sugar) on the survival and intestinal adhesion of a synthetic oral community exposed to an *in vitro* gastrointestinal digestión.

Methods

A synthetic biofilm of 14 species underwent to an *in vitro* digestion, in absence or presence of different food components (meat, oil, and sugar). Samples from oral, stomach and small intestine digestions were analyzed by flow cytometry to count live/dead cell. DNA was extracted from each digestion step and the community composition and structure was assessed by 16S rRNA amplicon sequencing. Bacterial adhesion was evaluated by adding 10^5-10^3 live bacteria/ml from the small intestinal digestion into a differentiated co-culture of Caco-2/HT29-MTX cells in a Transwell® system. Live/dead bacteria were quantified from the epithelial layer and the mucus niche by flow cytometry.

Conclusions

Meat had a protective effect towards bacterial survival, increasing the number of intact bacteria from 5 log units to 7 log units after gastrointestinal digestion. In addition, meat significantly supported the adhesion of bacteria to the small intestine mucosal surface (1.5 ± 0.1 log units) compared with control (1.3 ± 0.04 log units, $p < 0.001$). *Veillonella*, *Fusobacterium*, *Streptococcus* and *Aggregatibacter* were the genera with the highest relative abundances after the intestinal digestion. Structure and diversity were significantly different among communities co-digested with diverse food components.

The interaction between food and oral microbiota may affect the bacterial community survival, structure, and adhesion capacity to the small intestine. Further impact on host physiology remains to be elucidated.

FEMS7-2315
Environmental Microbiology/Microbial Ecology /Microbial Communities

DISPERSAL OR SELECTION, THE CASE OF ATMOSPHERIC MICROBIAL COMMUNITIES

J. Caliz[1], X. Triadó-Margarit[1], V. Jiménez-Ontiveros[2], D. Alonso[2], A. Àvila[3], E. O. Casamayor[1]
[1]Center for Advanced Studies of Blanes CSIC, Integrative Freshwater Ecology Group, Blanes, Spain
[2]Center for Advanced Studies of Blanes CSIC, Theoretical and Computational Ecology Laboratory, Blanes, Spain
[3]CREAF, Global Ecology Unit, Cerdanyola del Vallès, Spain

Backgrounds

The transport of particle-bound microorganisms over long distances throughout the atmosphere implies the annual mobilization around the planet on the order of 10 trillion of cells. Our knowledge on the processes that drive the atmospheric microbial communities is however still in its infancy. Airborne microorganisms are in constant interaction with human life, both directly as a source of pathogenic and beneficial microbes, and indirectly through biological effects on atmospheric processes. Most studies have suggested that changes on the composition of airborne microorganisms are related to the source of aerosols (dispersal) and selective processes mediated by environmental factors (environmental filtering). However, a long-term and well-tracked dataset has never been used to address these issues.

Objectives

The aim of this study is to determine the influence of dispersal and selection on atmospheric microbial communities.

Methods

In this study we applied high-throughput sequencing techniques (16S rRNA and 18S rRNA genes amplicons) to characterize the airborne microbial communities weekly collected in wet precipitations at La Castanya station (Montseny mountains, NE Spain, 41°46′N, 2°21′E), along 25 years.

Conclusions

We characterize the origin of air masses by modelled back trajectories and chemical composition of depositions. Depositions with non-ambiguous origin were used for microbial analyses to test whether the source of aerosols and/or the annual time period (related to environmental conditions) determine the composition and dynamics of airborne microbial communities.

FEMS7-2571
Environmental Microbiology/Microbial Ecology /Microbial Communities

STAPHYLOCOCCUS AUREUS SUPERNATANTS INHIBIT BIOFILM FORMATION IN CANDIDA GLABRATA

O. Camarillo-Marquez[1], C.H. Hernández-Rodríguez[1], A.V. Rodríguez-Tovar[1]
[1]National School of Biological Sciences-National Polytechnic Institute, Department of Microbiology, Ciudad de Mexico, Mexico

Backgrounds

It has been shown that with the presence of *Candida albicans* and *Staphylococcus aureus* significantly improves biofilm formation and its resistance to vancomycin, there is little information on the interaction of *Candida glabrata* and interaction with *S. aureus*.

Objectives

To standardize the formation of monospecies biofilms of *C. glabrata* and *S. aureus* and mixture of *C. glabrata-S. aureus* ,To determine the type of interaction between *C. glabrata* and *S. aureus*, Describe the ultrastructure of biofilms in co-culture.

Methods

To investigate the type of interaction between these microorganisms, the cells were examined in co-culture by scanning electron microscopy SEM, transmission electron microscopy TEM and confocal laser scanning microscopy CLSM.

Conclusions

It was evidenced the binding of *S. aureus* to the conidia of *C. glabrata*, the ultrastructure of the cells of *C. glabrata* had characteristic of dead cells, the same was observed with the CLSM the fungal cells were dead, but the cell wall was maintained intact. To demonstrate if cell-cell interaction was required, the free supernatants of *S. aureus* cells were obtained and they were found to inhibit the growth of *C. glabrata*, the mechanism of death is still unknown, a possible response is that the supernatants cause apotosis in fungal cells.

FEMS7-1139
Environmental Microbiology/Microbial Ecology /Microbial Communities

DOUBLE ACTION AGAINST DENTAL CARIES BY THE PROBIOTIC STREPTOCOCCUS DENTISANI

A. Camelo Castillo[1], A. López López[1], M. Ferrer Garcia[1], R. Cebrián Castillo[2], M. Martinez Bueno[2], A. Mira[1]
[1]Fundación para el Fomento de la Investigación Sanitaria y Biomédica FISABIO, Center for Advanced Research in Public Health. Genomics and Health Unit, Valencia, Spain
[2]University of Granada, Department of Microbiology, Granada, Spain

Backgrounds

A new Streptococcal species, isolated in our laboratory from tooth surfaces of caries-free humans, known as *Streptococcus dentisani sp.nov*, has been proposed to have a potential probiotic activity.

Objectives

Identify the mechanisms that *S.dentisani* uses to inhibit the oral pathogens and to buffer extracellular pH.

Methods

Using supernatants from *S. dentisani* cultures, we performed inhibition experiments against oral pathogens under solid and liquid media and we observed their antimicrobial effect using SEM.

By HPLC, MALDI-TOF-MS and the use of ion-exchange and hydrophobicity resins, the antibacterial compounds were purified from the supernatants of the probiotic culture. The growth and pH of *S. dentisani* in BHI medium enriched with L-arginine was measured for a 24h period, and the expression of arginolytic genes was measured by qPCR, performed in triplicates.

Conclusions

S.dentisani inhibits the growth of *S. mutans*, *S. sobrinus*, *Prevotella intermedia* and *F. nucleatum.* We have identified at least four matching peptide sequences of bacteriocins, in a region of about 16 kb, which we have called " Anti-Caries Defense Cluster" (ACDC).

S. dentisani buffers acidic pH in the presence of arginine, over-expressing genes for the production of ammonia when the pH becomes acidic.

We propose *S.dentisani* as a promising probiotic against tooth decay, with a double probiotic action, inhibiting the growth of major oral pathogens through the production of bacteriocins, and also buffering acidic pH (the primary cause of dental caries) through an arginolytic pathway.

FEMS7-2822
Environmental Microbiology/Microbial Ecology /Microbial Communities

CHARACTERIZATION OF SULFATE-REDUCING BACTERIA CONSORTIA AT ACIDIC CONDITIONS

N.G. Campos-Quevedo[1], E. Razo-Flores[2], N.E. Lopez-Lozano[2], I. Sánchez-Andrea[3], M.D.L.B. Celis Garcia[2]
[1]Instituto Potosino de Investigacion Cientifica y Tecnologica, Environmental Sciences, San Luis Potosi, Mexico
[2]Instituto Potosino de Investigación Científica y Tecnológica, Environmental Sciences, San Luis Potosi, Mexico
[3]Wageningen University, Microbial Physiology, Wageningen, The Netherlands

Backgrounds

Acid mine drainage (AMD) is an effluent generated from the weathering of mining waste and it causes several environmental problems. Sulfate reducing bacteria (SRB) are responsible for the production of sulfide which reacts with dissolved metals, forming precipitates (metal sulfides). However, the major drawback is that acetate cannot be degraded completely, and it can be toxic at the acidic conditions prevailing in AMD (pH 2-4).

Objectives

From the sequences analysis we aim to establish the potential novelty of some members that compose the consortia due to the fact that they are cultivable at lab-working conditions, and are able to consume acetate completely at acidic pH (4.0).

Methods

We managed to obtain 7 different cultivable consortia by traditional microbiology techniques (successive transfers), from a natural acidic environment (pH around 2). These consortia completely consume the substrate at acidic pH, either lactate or glycerol. Using molecular techniques (cloning-sequencing) it was possible to obtain the composition of the 7 cultivable consortia, which showed to have a great heterogeneity, although they were obtained from the same sediment, reflecting the effect of the culture conditions on the community.

Conclusions

From the data analysis we also could figure out the interactions between the community, and possibly identify new strains that could be isolated.

FEMS7-0524
Environmental Microbiology/Microbial Ecology /Microbial Communities

HYDROLASE-PRODUCING CHAOTOLERANT MICROBES ISOLATED FROM DIVERSE ENVIRONMENTS

P.E. Canales[1,2], *T.J. McGenity*[1]
[1]*School of Biological Sciences, University of Essex, Colchester, United Kingdom*
[2]*Laboratorio de Biología Molecular - Facultad de Farmacia y Bioquímica, Universidad Nacional Mayor de San Marcos, Lima, Peru*

Backgrounds

Chaotropic agents have the capability to disrupt biological systems like membranes, proteins and DNA. Chaotropicity has been studied to get more insights into the limits of life and, also, for its implications in fields like biotechnology, microbial ecology and astrobiology. Highly active enzymes under such extreme conditions are of interest for understanding cellular response and adaptation under chaotropic conditions, and even for potential industrial applications.

Objectives

The aim of this study was to determine the production of hydrolases by chaotolerant strains, compared under both chaotropic and kosmotropic conditions, isolated from diverse environments.

Methods

Samples included hypersaline and non-hypersaline environments: salt marsh (Colne Estuary, UK), agricultural soil (Colchester, UK) and Discovery deep-sea brine interface (Mediterranean Sea). Microbes belonged to the genera *Bacillus* sp., *Microbacterium* sp. and *Penicillium* sp. We compared the ability of chaotolerant microbes to produce hydrolases under both chaotropic (1 M $MgCl_2$) and kosmotropic (1 M NaCl) conditions using agar plate/tube assays supplemented with 1% yeast extract and the corresponding polymer for each test, except for the DNase test agar. Screening for proteases, amylases, lipases, cellulases, and DNases was carried out. One *Bacillus* sp. strain was able to produce a variety of extracellular enzymes (protease, lipase and DNase) under both chaotropic and kosmotropic conditions. Proteases able to degrade casein were produced in the highest number of strains (4 out of 5) under both conditions.

Conclusions

Diverse hydrolytic enzymes were produced under chaotropic conditions, but most chaotolerant microbes were able to grow faster and show higher production under kosmotropic than in chaotropic conditions.

FEMS7-2860
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE EXTREMES OF ROGOZNICA LAKE (EASTERN ADRIATIC COAST): IN-DEPTH CHARACTERISATION OF THE PROKARYOTIC COMMUNITY BENEATH THE OXIC ZONE

M. Čanković[1], G. Collins[2], E. Porca[2], I. Petrić[1], I. Ciglenečki[1]
[1]Institut Ruđer Bošković, Division for Marine and Environmental Research, Zagreb, Croatia
[2]School of Natural Sciences- NUI Galway, Microbiology, Galway, Ireland

Backgrounds

A large fraction of all prokaryotes is thought to inhabit anaerobic environments, which represent a pool of genetically diverse microorganisms. Earth's oxygen-depleted zones are expanding areas that are the site of, and are implicating in, changing biogechemical cycles. Under these conditions, microbial populations are essential mediators of elemental cycling and energy transfer. Rogoznica Lake, situated on the eastern Adriatic coast, and known as Dragon's Eye, is a highly eutrophic marine lake. With no fresh water inflow or drifts, it is considered a self-sustaining environment. This uniquely extreme ecosystem is euxinic (i.e. sulfidic) and meromictic. Due to its steep physico-chemical gradient (particularly oxygen and sulfide) it provides a suitable environment for the development of microaerophilic and anaerobic prokaryotic communities.

Objectives

Although extensively studied, nothing is yet known about the microbial community living beneath the oxic zone in Rogoznica Lake.

Methods

We investigated the spatio-temporal dynamics of the active bacterial and archeal communities by sequencing of 16S rRNA amplicons generated from cDNA.

Conclusions

Clear differences in the structure of the active community were observed across the vertical profile as well as during different seasons, all of which corresponded closely to environmental parameters. A complex and diverse distribution of microbial populations was revealed, supporting the idea that anaerobic degradation of organic matter in the Lake is driven by a range of taxa adjusted to environmental constraints.

FEMS7-0930
Environmental Microbiology/Microbial Ecology /Microbial Communities

WILD GRAPE-ASSOCIATED YEASTS AS A PROMISING STRATEGY OF BIOCONTROL AGAINST VITIS VINIFERA FUNGAL PATHOGENS

C.B. Gustavo[1], V. Ileana[2], F. Roberto[2], M. David[3], M. Nicola[2], J.M. Cantoral Fernandez[1]
[1]Universidad De Cadiz, Biomedicina- Biotecnologia Y Salud Publica. Lab. Microbiologia, Puerto Real, Spain
[2]Universita Degli Studi Di Milano, Department Of Food- Environmental And Nutritional Sciences, Milano, Italy
[3]Agricultural University Of Georgia, Institute Of Horticulture- Viticulture And Oenology, Tbilisi, Georgia

Backgrounds

The increasing level of hazardous residues in the nature and food has led to the European Union to restrict the use of chemical fungicides, addressing the researchers to face this challenge. Exploiting new natural antagonistic microorganisms against fungal diseases could serve agricultural production reducing pre- and postharvest losses.

Objectives

The main aim of this work was to isolate epiphytic and endophytic yeasts from grape berries collected from *Vitis vinifera* ssp *sylvestris* populations in the Mediterranean Basin and from *Vitis vinifera* ssp *vinifera* cultivars in three different farming systems: organic, biodynamic and conventional.

Methods

Strains able to prevent fungal pathogenic attacks of grape-berries were selected for in *vitro* and in *vivo* antagonism experiments against *Botrytis cinerea*, *Aspergillus carbonarius* and *Penicillium expansum* species. The more effective antagonist yeast strains were subsequently assayed for their capability to colonize and grow in grape wounds. Finally, the possible modes of action: nutrients and space competition, iron depletion, cell-wall degrading enzymes, diffusible and volatile antimicrobial compounds, and biofilm formation, were investigated as well.

Conclusions

245 yeasts strains belonging to 8 species were isolated, 20 of them showed antagonistic action against all molds. Yeast strains isolated from grape-berries from *V. vinifera* ssp *sylvestris* were more effective (up to 60%) against *B. cinerea* rather than other strains isolated from *V. vinifera* ssp *vinifera*. Six antagonistic yeasts, all isolated from wild vines, belonging to 4 species (*Meyerozyma guilliermondii*, *Hanseniaspora uvarum*, *Hanseniaspora clermontiae* and *Pichia kluyveri*) revealed phenotypical characteristics associated to the analysed modes of action.
Project founded by Talent-Hub COFUND-GA_nº291780 & IRSES_GA_n°612442

FEMS7-1815
Environmental Microbiology/Microbial Ecology /Microbial Communities

GUT MICROBIOME AND SOCIAL COOPERATION IN DROSOPHILA MELANOGASTER

P. Carazo[1], R. García-Roa[1], A. Bartolomé[1], L. Bartolomé[2], A. Latorre[3]
[1]Cavanilles Institute of Biodiversity and Evolutionary Biology, Ecology- Ethology and Evoltion Group, Valencia, Spain
[2]Science and Technology Faculty- the Basque Country University/EHU, Central Analytical Service SGIker, Bilbao, Spain
[3]Institute for Integrative Systems Biology, Evolutionary Systems Biology of Symbionts, Valencia, Spain

Backgrounds

Understanding the evolution and maintenance of cooperation is an enduring question in biology. Kin selection favours the appearance of adaptations that foster cooperation between related individuals, and has proven to be a fundamental process in the evolution and maintenance of cooperation, and of social evolution at large. Kin selection frequently relies on the ability to recognize kin from non-kin and recent evidence suggests that, by modifying body odours, gut microbiota might be crucially involved in kin recognition in species that largely rely on olfaction.

Objectives

In this study, we assess the role of the gut microbiota in kin recognition in *Drosophila melanogaster*. Recent studies have shown that kin recognition mediates a variety of social cooperative behaviours in this species, but the mechanisms underlying kin recognition remain poorly understood.

Methods

Here, we studied the relative effects of genotype (i.e. family) and rearing environment (i.e. diet) in determining the gut microbiome of flies, how variation at this level translates into variation in body odours (i.e. cuticular hydrocarbons; CHCs), and how this in turns affects kin recognition and social cooperation.

Conclusions

Our results have important bearing to understand the evolution of social cooperation in *D. melanogaster*, and more generally the role of gut microbes in kin recognition in insects.

FEMS7-0413
Environmental Microbiology/Microbial Ecology /Microbial Communities

EEL MICROBIOME REVEALS THAT SKIN MUCUS OF FISH IS A NATURAL NICHE FOR AQUATIC INTESTINAL PATHOGEN EVOLUTION

M. Carda-Diéguez[1], R. Ghai[2], F. Rodríguez-Valera[3], C. Amaro[1]
[1]Universitat de Valencia, Microbiología y Ecología. ERI Biotecmed, Burjassot, Spain
[2]Institute of Hydrobiology- Biology Center of the Academy of Sciences of the Czech Republic, Department of Aquatic Microbial Ecology, České Budějovice, Czech Republic
[3]University of Miguel Hernández, Evolutionary Genomics Group- Producción Vegetal y Microbiología, San Juan de Alicante, Spain

Backgrounds

Accidental pathogens of aquatic origin such as vibrios evolve in their natural environment usually by horizontal gene transfer events. We hypothesized that fish's skin-mucosal-surface (SMS) can act as a niche that accelerates and favors the emergence of new accidental intestinal pathogens in the environment.

Objectives

i) To determine the SMS's microbiome, virulome, resistome and mobilome from eels and the differences from that of the surrounding water (W); ii) to describe the specific functionalities associated to SMS-microbiome (mucus-signature); iii) to sequence isolated opportunistic pathogens present in the SMS and compare them with the genomes of true pathogenic isolates.

Methods

We have determined the SMS- and water-microbiome by using metagenomic and bioinformatic tools. Intestinal pathogens were cultured, identified by phenotypic and genotypic tests, sequenced and compared with that deposited in the databases.

Conclusions

Vibrio species monopolized wild-eel's SMS-microbiome from natural ecosystems. *V. anguillarum/V. vulnificus* and *V. cholerae/V. metoecu*s being the most abundant ones in SMS from estuary and lake, respectively. Functionalities of the SMS-microbiome differed significantly from those of W-microbiome and suggested that colonizers should contain specific genes for attachment, bacterial competition and communication, and resistance to mucosal innate immunity, predators and heavy metals/drugs. Furthermore, some of these genes were associated to mobile genetic elements, mainly Integrative and Conjugative Elements. Finally, the genomic comparisons of a *V. metoecus* isolate from SMS presented characteristics intermediate between *V. metoecus* from water/extra-intestinal infections and *V. cholerae* O1 from cholera patients suggesting that HGT events between close *Vibrio* species could take place in this mucosal environment.

FEMS7-1105
Environmental Microbiology/Microbial Ecology /Microbial Communities

ANALYSIS OF THE MOLECULAR MACHINERY IMPLICATED IN MULTICELLULARITY IN BACILLUS CEREUS

J. Caro Astorga[1], D.F. Romero Hinojosa[1], A. De Vicente Moreno[1], E. Frenzel[2], O. Kuipers[2]
[1]Facultad de Ciencias., Microbiologia, Málaga, Spain
[2]Groningen Biomolecular Sciences and Biotechnology Institute GBB.,
2Department of Molecular Genetics. University of Groningen, Groningen, The Netherlands

Backgrounds

Bacillus cereus is a Gram-positive bacterium usually implicated in food poisoning outbreaks and human infections that sometimes result fatal. These events are closely related to the assembly of a biofilm that serves as a reservoir of cells, a nest for sporulation and protection from environmental stresses, host defenses or chemotherapy.

Objectives

To perform a comprehensive comparative study of biofilm and planktonic cells to: i) delineate the molecular machinery implicated in the different steps of the biofilm life cycle, and ii) define new genes dedicated to biofilm formation.

Methods

Bacteria were grown under biofilm inducing conditions. Biofilm cells were separated from planktonic cells at different times and their whole mRNA was isolated, sequenced and analyzed.

Conclusions

Our results reveal a high number of genes associated to biofilm, many of them with unknown function, but highly conserved in others bacterial species. Besides, we found global changes in cell wall synthesis, metabolism and interspecies interaction molecules. The interaction of *B. cereus* with other bacteria is conditioned by secondary metabolites, which are apparently overexpressed in biofilm. On the other hand, toxins are mainly expressed in planktonic cells, which are more oriented to interact with its hosts. These results reveal the defense and attacking positions of *B. cereus* in biofilm vs planktonic states.

This work was supported by grants AGL-2012-31968 and ERC-Starting Grant BacBio ERC637971. Joaquin Caro-Astorga is supported by a PhD fellowship from the FPI program of the Spanish Government.

FEMS7-1053
Environmental Microbiology/Microbial Ecology /Microbial Communities

GENETIC DIVERSITY AND ANTIMICROBIAL SUSCEPTIBILITIES OF NOCARDIA CYRIACIGEORGICA STRAINS ISOLATED FROM SOIL

G. Carrasco[1], M. Sansegundo[1], M.A. Fernández[1], A.C. Ramírez[2], E. García-Amor[2], N. Garrido[1], P. Villalón[1], J.A. Saez-Nieto[1], S. Valdezate[1]
[1]National Centre of Microbiology / Institute of Health Carlos III,
Taxonomic Reference and Research Laboratory, Majadahonda Madrid, Spain
[2]Faculty of Pharmacy and Bioanalysis- Los Andes University,
Laboratory of Soil Microbiology. Department of Microbiology and Parasitology, Mérida, Venezuela

Backgrounds

Nocardia genus is a gram-positive rod present in soil, which shares ecological habitat with other *Actinomycetes* as *Streptomyces* spp. From soil, *Nocardia* spp. infection is mostly acquired by humans via inhalation or after skin injury.

Objectives

Comparison of the diversity of *N. cyriacigeorgica* soil population and their susceptibilities respect to human strains.

Methods

Twenty-eight *N. cyriacigeorgica* strains isolated from the soil (100m^2/10cm-depth) of nine areas of Lara state (Venezuela) were compared to 30 human strains from Spain. 16S rRNA, *gyrB* full genes and 16S-*gyrB-hsp65-secA1* concatenated MLSA (McTaggart *et al*., 2010) sequences were analyzed. Antimicrobial susceptibilities were undergone by microdilution method with RAPMYCO panels (Thermo-Scientific). MIC values were categorized following Clinical Laboratory Standard Institute interpretative criteria (CLSI, 2011).

Conclusions

N. cyriacigeorgica was the major *Nocardia* species obtained in the studied Venezuelan soil (71.8%). Respect to the human strains, higher variability was found in the 16S rRNA gene sequence in soil strains ($HGDI_{soil}$ 0.57 vs. $HGDI_{human}$ 0.0), but lower in *gyr*B (0.79 vs. 0.94). The phylogenetic MLSA tree of soil strains clustered the main *gyr*B haplotype (n=13) in 12 clades. The soil strains exhibited resistance rates up to 25% for amoxicillin-clavulanic acid, cefoxitin, fluoroquinolones, clarithromycin and doxycycline. Differences in MIC distribution and resistance rates were found among soil and human strains, being the soil strains more susceptible. Statistical differences *($p \leq 0.05$)* were found with amoxicillin-clavulanic acid, third generation cephalosporins, imipenem, fluoroquinolones and minocycline**.** Our study gives insight into unreported diversity and susceptibility of soil *N. cyriacigeorgica* strains, one of the main agent of human nocardiosis.

FEMS7-0757
Environmental Microbiology/Microbial Ecology /Microbial Communities

BRIDGING THE GAP BETWEEN EMPIRICAL MICROBIAL ECOLOGY AND THEORIES OF BIODIVERSITY ORGANIZATION IN SPACE AND TIME

E.O. Casamayor[1], D. Alonso[1], J.A. Capitan[2], X. Triadó-Margarit[1], J. Caliz[1], R. Ortiz-Alvarez[1], V. Jimenez-Ontiveros[1]
[1]Center for Advanced Studies of Blanes CSIC, Continental Ecology, Blanes, Spain
[2]UNIVERSIDAD POLITÉCNICA DE MADRID, DEPARTAMENTO DE MATEMÁTICA APLICADA, Madrid, Spain

Backgrounds

Most ecological theory has been developed for plants and animals. However, for the last years, technical progress on several fields, from computers to last generation DNA sequencers, has made it possible to collect large amounts of data on microbes at unprecedented rates. Microbial systems are also easy to sample massively and expand along orders of magnitude of environmental factors. All these facilities makes microbial communities excellent model systems to check general ideas on the function and structure of ecological communities and expand ecological theory.

Objectives

Most recent work on microbial communities describes spatial patterns, such as the species area relationship and beta diversity indexes, or take statistical approaches to describe snapshots of communities that are assumed to be at a steady state. Although temporal variation is fully appreciated and described for microbial communities, few attempts have been done yet to study the leading mechanisms driving community assembly in time. We will explore the potential of classic island biogeography theory to gain insights into the temporal dynamics and regional scale distribution of microbial communities.

Methods

A stochastic model formulation of the theory is used as a way to estimate an equivalent of effective colonization and extinction rates per microbial group, bridging the gap between empirical microbial ecology and theories of biodiversity organization in space and time.

Conclusions

We carried out a first attempt to describe microbial communities as the result of individual extinction/colonization stochastic events acting on an ensemble of bacterial populations belonging to a regional pool. The approach provided a powerful starting point to study temporal community re-assembly after environmental perturbations.

FEMS7-0764
Environmental Microbiology/Microbial Ecology /Microbial Communities

CLIMATE CONTROL OF REMOTE MICROBIAL DISPERSAL THROUGH ATMOSPHERIC AEROSOLS: A LONG-TERM STUDY IN THE CENTRAL PYRENEES (LTER-AIGUESTORTES)

E.O. Casamayor[1], *X. Triadó-Margarit*[1], *J. Caliz*[1], *R. Ortiz-Alvarez*[1]
[1]*Center for Advanced Studies of Blanes CSIC, Continental Ecology, Blanes, Spain*

Backgrounds

Dust coming from the large deserts on Earth, such as the Sahara, can travel long distances and be dispersed over thousands of square kilometers. Remote dust deposition rates are increasing as a consequence of global change and may represent a mechanism for intercontinental microbial dispersal. Remote oligotrophic alpine lakes are particularly sensitive to dust inputs and can serve as sentinels of airborne microbial transport and the ecological consequences of accelerated intercontinental microbial migration.

Objectives

Microbes are extremely abundant and ubiquitous on Earth, with a huge genetic and metabolic diversity and a large ability to quickly react to any change in the environmental conditions. They are therefore excellent sensors of environmental changes and one of the main actors in the ecosystems response. Our investigations carried out in the Aigüestortes National Park within the Spanish Long Term Ecological Research network (LTER), want to determine the role of terrestrial aerosols as a natural mechanism for global dispersal and ubiquity of microorganisms.

Methods

We applied high-throughput sequencing techniques (16S rRNA and 18S rRNA amplicon sequencing) to characterize the airborne microbial communities reaching the Central Pyrenees (NE Spain) along a long term study, and the planktonic microorganisms of the lacustrine Pyrenean district.

Conclusions

Overall, this study suggests that local and regional features may generate global trends in the dynamics and distribution of airborne microbial assemblages, and the diversity of viable cells in the high atmosphere is likely higher than previously expected.

FEMS7-0329
Environmental Microbiology/Microbial Ecology /Microbial Communities

ISOLATION, CHARACTERIZATION AND SELECTION OF BACTERIAL ISOLATES FROM A SUPPRESSIVE SOIL WITH BENEFICIAL TRAITS TO PLANTS

C. Vida[1], A. de Vicente[1], S. Tienda[1], J.A. Gutierrez-Barranquero[1], F.M. Cazorla[1]
[1]Facultad de Ciencias- Universidad de Málaga, Microbiología, Málaga, Spain

Backgrounds

This study focused on the characterization and selection of bacterial strains obtained from a suppressive soil displaying antifungal activity against the soilborne phytopathogenic fungi *Rosellinia necatrix*. Bacterial profile from this suppressive soil were first obtained by 16S rRNA gene sequencing, revealing a significant increase in the bacterial class *Gammaproteobacteria*, especially in some antagonistic representatives of *Pseudomonas* spp.

Objectives

To obtain and characterize a collection of 246 bacterial isolates obtained from this suppressive soil, in order to identify new strains with antifungal activity against fungal phytopathogens.

Methods

To obtain the bacterial collection, we performed an isolation on a selective medium for *Pseudomonas*-like microorganisms. Further characterization tests were used in order to analyse the bacterial collection, including identification of the general metabolic profile of glucose, the profiling of antifungals produced, including both the putative production of antifungal compounds and lytic exoenzymes, and the evaluation of traits related with beneficial effects on plants.

Conclusions

A final selection of representative strains resulted in antifungal isolates belonging to the genus *Pseudomonas,* but also some representatives of the genera *Serratia* and *Stenotrophomonas.* These selected strains were tested for plant protection by an *in vivo* experiment using avocado and wheat plants challenged by the pathogen *R. necatrix*, showing all of them an antifungal ability and plant disease protection.
Pseudomonas-like strains isolated from suppressive soils constitute an excellent source for novel microbial biocontrol agents against soilborne fungal pathogens.

This work was supported by grant AGL2014-52518-C2-1-R. Carmen Vida and Sandra Tienda are supported by a PhD fellowship from the FPI program of the Spanish Government.

FEMS7-0969
Environmental Microbiology/Microbial Ecology /Microbial Communities

ASSESSMENT OF THE PROKARYOTIC BIODIVERSITY OF THE SALTERNS OF MARGHERITA DI SAVOIA (ITALY) BY AMPLICON BASED NEXT GENERATION SEQUENCING ANALYSIS

L.R. Ceci[1], C. Leoni[2], M. Volpicella[2], C. Manzari[1], F. Bruno[1], P. Elisabetta[2], G. Bruno[1], P. Graziano[1,2]
[1]Institute of Biomembranes and Bioenergetics, CNR, Bari, Italy
[2]Department of Biosciences- Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy

Backgrounds

Marine salterns are excellent sites for studying the dynamics of the prokaryotic biodiversity at increasing salt concentrations. Metagenomics offers the most direct approach for reliably assessing the microbial diversity expecially for uncultivable prokaryotes.
Salterns of Margherita di Savoia (MdS) are located on the East-cost of South Italy. They are the largest salterns in Europe, with a yet unexplored microbiota composition.

Objectives

Defining the microbiota composition of the salterns of MdS in ponds with increasing salt concentrations.

Methods

eDNA purified from nine ponds with salt concentration in the 4.9-36% range was used for PCR amplification of the V5-V6 hypervariable region of the 16S rRNA gene. NGS of amplicon libraries was carried out by the Illumina MiSeq platform. Obtained reads were analyzed using the BioMaS software for taxonomic classification (Fosso et al., 2015, BMC Bioinformatics).

Conclusions

The microbiota composition of the MdS salterns resulted in a peculiar composition of prokaryotes, quantitatively different from that of other investigated salterns of the Mediterranean area. For example, Archaea are absent at low salt concentrations (4.9-8.4%) and reach their highest concentrations (30-35%) in the high-salinity ponds. In similar ponds of the salterns of Santa Pola (Spain) their presence has been estimated around 90%. Conversely, in the high-salinity ponds of the MdS salterns, the Eubacteria *Salinibacter* genus is the most represented genus.
This study is of particular interest, not only to define the microbiota composition in different salt concentrations, but also for better addressing future functional metagenomics analysis aimed at the identification of biotechnological useful extremozymes.

FEMS7-2257
Environmental Microbiology/Microbial Ecology /Microbial Communities

DIVERSITY OF SALMONELLA SPP. CIRCULATING IN WILD ENDEMIC LIZARDS IN NATURAL AND SEMI-URBAN ENVIRONMENTS

E. Gómez-Díaz[1], T. Ayats[2], J. Huertas[3], J. Figuerola[1], L. Santamaría[1], M. Cerdà-Cuéllar[2]
[1]Estación Biológica de Doñana, CSIC, Sevilla, Spain
[2]IRTA-CReSA, Research Center on Animal Health, Bellaterra, Spain
[3]IBE Institut de Biologia Evolutiva, CSIC-UPF, Barcelona, Spain

Backgrounds

Wild animal populations contribute significantly to the transmission, spread and persistence in the environment of a number of bacterial pathogens, such as *Salmonella*. However, little is known about the circulation and diversity of *Salmonella* in wild, free-living species.

Objectives

Here, we studied the occurrence and genetic diversity of *Salmonella* spp. from two lizard species (*Podarcis pityusensis* and *Podarcis lilfordi*) from the Balearic Archipelago, a touristic hotspot in the Mediterranean. These two lizards are highly abundant and distribute in dense populations that are in close contact with humans, providing a likelihood of human infection.

Methods

A total of 197 fecal samples were analyzed (4 populations and 2 years), and *Salmonella* detection was performed both by PCR and conventional culture methods. Isolates were characterized by serotyping and Multilocus Sequence Typing (MLST).

Conclusions

Overall, 46 samples tested PCR-positive. Among these, *Salmonella* was isolated from 41. There were not clear differences in prevalence between species or sexes or geographic structuring between semi-urban and natural areas. There was a wide diversity of subspecies and serovars, with isolates from all six subspecies (*enterica, salamae, arizonae, diarizonae, houtenae, indica*) and five serovars within supbsp. *enterica*. Together with this phenotypic diversity, a variety of MLST sequence types (ST) was found, with only three already described ST (508, 958, 2635) and up to 12 undescribed new ST. These findings illustrate the role of lizards as reservoir of *Salmonella* spp*.,* with some of them being pathogenic to humans, and highlight the need to closely monitor the wildlife-livestock-human interface in these touristic areas for disease prevention and wildlife conservation.

FEMS7-2490
Environmental Microbiology/Microbial Ecology /Microbial Communities

IS THERE A MICROBIOLOGICAL RISK IN RECREATIONAL ACTIVITIES WITH DROMEDARIES?

S. Vega[1], S. Sevilla-Navarro[1], M. Cerdà-Cuéllar[2], T. Ayats[2], C. Marín[1]
[1]Universidad CEU-Cardenal Herrera,
Departamento de Producción y Sanidad Animal- Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Alfara del Patriarca -Valencia, Spain
[2]IRTA, CReSA Research Center on Animal Health, Bellaterra, Spain

Backgrounds

Campylobacter and *Salmonella* are the main cause of food-borne enteritis in the European Union (UE). Wild animals can act as reservoirs and vehicles of transmission of these zoonotic agents. In Canary Islands (Spain) there is the most important population of dromedary camels (*Camelus dromedarius*) of the UE. These animals are exported to other countries and have direct contact with humans. Thus, there is a need to study their role in zoonotic bacteria dissemination.

Objectives

The aim of this study was determine the occurrence of *Campylobacter* and *Salmonella* in dromedary camels from Canary Islands and to study the genetic diversity of the isolates.

Methods

During February 2016, 54 camels were sampled in Tenerife (Canary Is.). Cloacal swabs were obtained for *Campylobacter* isolation, whilst faeces were collected directly from the rectum for *Salmonella* isolation. Samples were analysed according to ISO10272-1: 2006 (Annex E) for *Campylobacter* and ISO 6579: 2002 (Annex D) for *Salmonella.* Genetic diversity of the isolates was studied by pulsed field gel electrophoresis (PFGE).

Conclusions

None of the samples were *Campylobacter* positive. Three out of 54 camels (5,5%) were positive for *Salmonella* spp. All isolates belonged to serovar Frintrop and showed an identical or nearly identical PFGE profile (> 96% similarity).

Dromedaries from Canary Is. are therefore asymptomatic carriers of *Salmonella* spp. but not of *Campylobacter*. The presence of *Salmonella* in these animals may constitute a health hazard to humans. Biosafety procedures should be therefore implemented in animals to be transported through different countries and in recreational activities, to avoid zoonotic infection.

FEMS7-1351
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECTS OF BACTERIAL INOCULATION ON THE RHIZOREMEDIATION POTENTIAL OF POPULUS CV. SKADO GROWING IN SOILS CO-CONTAMINATED WITH POLYCYCLIC AROMATIC HYDROCARBONS (PAH) AND TRACE METALS

A. Cerdeira-Pérez[1], B. Rodriguez-Garrido[1], Á. Prieto-Fernández[1], P.S. Kidd[1], M. Chalot[2]
[1]Instituto de Investigaciones Agrobiológicas de Galicia IIAG- Consejo Superior de Investigaciones Cie ntíficas CSIC, Soil Biochemistry, Santiago de Compostela, Spain
[2]Université de Bourgogne Franche-Comté, Laboratoire Chrono-Environnement, Montbéliard, France

Backgrounds

Polycyclic aromatic hydrocarbons (PAH) are one of the most ubiquitous persistent organic pollutants in soils with potential adverse effects on human health and the environment. Many sites are affected by co-contamination of mixtures of different PAH and toxic concentrations of trace metals. Rhizoremediation, which exploits the interactions between plants and their associated microorganisms to degrade organic compounds, is one of the more promising strategies for the treatment of PAH-contaminated soils.

Objectives

The aim of this study was to identify potentially useful plant-bacterial partnerships for application in the remediation of trace metal- and PAH- contaminated soils.

Methods

For this, several rhizobacterial and root endophytic strains were screened at a bench-scale in order to select those with highest potential to dissipate and/or mobilize different PAH compounds. Bacterial proliferation, degradation capacity and biosurfactant production were assessed in culture media. Molecular assays characterized bacterial strains for the presence of PAH ring-hydroxylating dioxygenase genes. Furthermore, greenhouse experiments based on bacterial inoculation of *Populus* cv. Skado growing on two substrates with organo-metallic contamination were carried out in order to identify those bacterial strains with greater plant growth promotion capacity. Plant tolerance, biomass production, nutritive status and stress enzyme activities were determined, as well as the effect of the plant-bacterial system on PAH dissipation and metal mobility.

Conclusions

On the basis of the results obtained several strains were identified presenting a combination of useful traits for use as bioinoculants in rhizoremediation techniques targeting PAHs, or which could be used in combination as a consortium in future research in this field.

FEMS7-2881
Environmental Microbiology/Microbial Ecology /Microbial Communities

DIVERSITY OF ADHERENT-MICROBIAL COMMUNITIES IN COLON MUCOSAL BIOPSIES FROM CHILEAN AND SPANISH ULCERATIVE COLITIS AND CROHN´S DISEASE PATIENTS

N. Chamorro Veloso[1], R. Rosselló-Móra[2], M. Hermoso[3], R. Vidal[1]
[1]Facultad de Medicina -Universidad de Chile, MICROBIOLOGY, Santiago, Chile
[2]Institut Mediterrani d'Estudis Avançats IMEDEA- CSIC-UIB,
Department of Ecology and Marine Resources, Esporles, Spain
[3]Facultad de Medicina -Universidad de Chile, Programa Disciplinario de Inmunología, Santiago, Chile

Backgrounds

Dysbiosis has been associated with changes in the individual's diet, antibiotic use and a number of clinical disorders, including inflammatory bowel diseases (IBDs). Mostly identified microbiome adhered to the mucosa has been made using short sequences (<300 nucleotides) difficulting clear affiliation at the genus or species level. In this study, we amplified 16S rRNA sequences belonging to biopsies of Chilean Ulcerative Colitis (UC), Crohn's Disease (CD) patients and control individuals using high quality reads (>300 nucleotides) for affiliation process

Objectives

To characterize the microbiota of colonic mucosa obtained from Chilean IBD patients and compare with previous studies performed in Spanish IBD patients

Methods

16S rRNA gene partial sequences were amplified from biopsies taken from 20 UC, 21 CD patients and 9 healthy subjects, by 454 GS FLX Plus System. The phylogenetic inferences from reads were grouped in OPU through ARB software

Conclusions

IBD patients can be classified in different groups according to their microbial composition. IBD1 group presented predominance in the *Bacteroidetes* proportion (40%) while IBD2 group showed a predominance in *Proteobacteria* (57%). Moreover, an IBD3 group exhibited *Actinobacteria* predominance, while IBD5 group, included only Chilean samples showing a *Proteobacteria* predominance (70%) with a large proportion of *Klebsiella oxytoca*. Furthermore, Chilean´s IBD and Spanish control samples were grouped together in IBD4, with a *Firmicutes* (65%) predominance characterized by Clostridia abundance. In conclusion, IBD patients are not distributed in a single homogenous group. In addition, Chilean IBD samples have a microbiota more similar to healthy patients.

FEMS7-0528
Environmental Microbiology/Microbial Ecology /Microbial Communities

NITROGEN REMOVAL PERFORMANCE AND MICROBIAL COMMUNITY OF A MULTISTAGE A/O BIOFILM PROCESS FOR LOW COD WASTEWATER TREATMENT

H. Chen[1], A. Li[1], F. Ma[1], C. Cui[1], H. Zhu[1], Q. Wang[1]
[1]Harbin Institute of Technology, State Key Laboratory of Urban Water Resource and Environment, Harbin, China

Backgrounds

Biological nitrogen removal has been widely applied in wastewater treatment for decades with various advantages. However, deficiency of organic carbon in low COD wastewater usually leads to deteriorative nitrogen removal and unstable biomass retention.

Objectives

A novel multistage A/O biofilm reactor was developed to improve the nitrogen removal from low COD wastewater in this study.

Methods

Polyurethane carriers with domesticated nitrification biofilm and denitrification biofilm were filled respectively in bench-scale reactor as multistage A/O mode to optimize the utilization of limited organic carbon. Key parameters were investigated to obtain optimal performance, and high-throughput sequencing technology was adopted to reveal the microbial community structure in biofilm process.

Conclusions

The results show that the optimal values of key parameters including nitrification liquid reflux ratio, hydraulic retention time and influent flow distribution ratio were 200%, 8 h and 2:1, respectively. Best ammonium and total nitrogen removal efficiency of multistage A/O biofilm process treating low COD wastewater could respectively reach 99% and 40%, while the effluent ammonium and total nitrogen were only 0.1 mg/L and 8 mg/L. Microbial community analysis in multistage A/O biofilm reactor showed that *Nitrosomonadaceae_uncultured* and *Nitrospira* were dominant responsible genera in nitrification biofilm, while genera with nitrate reduction ability including *Thauera*, *Flavobacterium* and *Pseudomonas* were dominant in denitrification. Functional microbial consortia in domesticated biofilm could steadily colonize and reproduce in the process along with a predominant nitrogen removal performance. Multistage A/O biofilm process shows promising performance for low COD wastewater treatment, which provides a choice for further application in practical conditions.

FEMS7-2947
Environmental Microbiology/Microbial Ecology /Microbial Communities

EXPRESSION OF THE PHOSPHODIESTERASE BIFA FACILITATING SWIMMING MOTILITY IS PARTLY CONTROLLED BY FLIA IN PSEUDOMONAS PUTIDA KT2440

W. Chen[1], *H. Qiaoyun*[1]
[1]*Huazhong Agricultural University, State Key Laboratory of Agricultural Microbiology, Wuhan, China*

Backgrounds

Flagella-mediated motility is an important capability of many bacteria to survive in nutrient-depleted and harsh environments. Decreasing the intracellular cyclic di-GMP (c-di-GMP) level by overexpression of phosphodiesterase enzyme BifA promotes flagellar-mediated motility and induces a planktonic lifestyle in *Pseudomonas* species. The mechanism that regulates the expression of *bifA* gene was poorly studied.

Objectives

The flagellar sigma factor, FliA control numerous genes other than those involved in flagellar biogenesis. The transcription of *bifA* in a *P. putida fliA* mutant decreased approximately two fold (Rodríguez-Herva *et al.*, 2010). Therefore, we hypothesized that the expression of BifA was putatively directly regulated by FilA and FliA possessed an ability to modulate intracellular c-di-GMP level via regulating BifA expression in *P. putida*.

Methods

Swimming motility assay, biofilm formation analysis and quantification of intracellular c-di-GMP were carried out to test the hypothesis.

Conclusions

The expression of BifA was partly controlled by FliA (σ^{28}) in *P. putida* KT2440. FliA deletion led to about twofold decrease in transcription of *bifA*. 5' race assay revealed two transcription start points in *bifA* promoter region, with the putative σ^{70} and σ^{28} promoter sequences upstream, respectively. FliA overexpression decreased the intracellular c-di-GMP level in a BifA dependent way, suggesting that FliA could modulate intracellular c-di-GMP level and BifA function was required for the modulation. Besides, FliA overexpression enhanced swimming ability of wild type strain, while made no difference to the *bifA* mutant. Our results suggest that FliA acts as a negative regulator to modulate c-di-GMP level via controlling transcription of *bifA* to facilitate swimming motility.

FEMS7-0850
Environmental Microbiology/Microbial Ecology /Microbial Communities

POPULATIONS DYNAMICS AND TRANSCRIPTOMIC RESPONSE OF PSEUDOMONAS AERUGINOSA TOWARDS ANTIBIOTICS IN 19 SPECIES PLANKTONIC AND BIOFILM MICROBIAL COMMUNITIES

Y.Y. Cheng[1], J.K.H. Yam[1], Z.J. Seng[1], L. Yang[1]
[1]Singapore Centre for Environmental Life Sciences Engineering SCELSE,
Singapore Centre for Environmental Life Sciences Engineering SCELSE, Singapore, Singapore

Backgrounds

Pseudomonas aeruginosa is an opportunistic pathogen responsible for a wide range of human and animal infections. *P. aeruginosa* is notorious for its antibiotic resistance and previous research has revealed several important mechanisms of this, including the activities of efflux pumps and biofilm formation. However, as a metabolically versatile bacterium, *P. aeruginosa* is able to adapt to various environments and often lives together with other microbial species. It remains largely unknown how *P. aeruginosa* adapt to antibiotic stress in a mixed-species community.

Objectives

To investigated the population dynamics and global stress response of *P. aeruginosa* towards tobramycin and colistin in 19 species planktonic and biofilm microbial communities.

Methods

We found that *P. aeruginosa* is able to gain fitness over other species in biofilm community while not in planktonic community, independent of the presence of antibiotics. RNA-seq based transcriptomic analysis showed that biofilm *P. aeruginosa* cells strongly induced the expression of quorum sensing regulated genes and stress response genes compared to planktonic *P. aeruginosa* cells in the 19 species communities without the presence of antibiotics.

Conclusions

Our study suggests that *P. aeruginosa* possess competitive advantage relative to other microbial species especially in biofilm mode of growth, which might lead to increase in its virulence and antibiotic resistance.

FEMS7-0734
Environmental Microbiology/Microbial Ecology /Microbial Communities

BOTTLENOSE DOLPHINS AND KILLER WHALES HARBOR A UNIQUE SKIN MICROBIOME THAT VARIES AMONG INDIVIDUALS

M. Chiarello[1], T. Bouvier[2], S. Villeger[2], C. Bouvier[2], J.C. Auguet[2]
[1]Université de Montpellier, UMR9190 MARBEC, Montpellier, France
[2]CNRS, UMR9190 MARBEC, Montpellier, France

Backgrounds

Marine animals host diverse and species-specific microbial communities on their skin that could play a major role for their health. However, most inventories of marine animals skin microbiomes have focused on corals and fishes, while the microbiome of cetaceans remains overlooked.

Objectives

Few studies have focused on wild or semi-captive cetaceans, making difficult to distinct intrinsic inter- and/or intraspecific variability in skin microbiomes from environmental effects. Assessing inter- and intraspecific variability of cetaceans skin microbiome in controlled conditions is also needed to better understand the differences with skin microbiome of other marine vertebrates and terrestrial mammals.

Methods

Here, using high-throughput sequencing, we assessed the skin microbiome of 8 individuals of two emblematic species, the bottlenose dolphin (*Tursiops truncatus*) and the killer whale (*Orcinus orca*), housed in captivity in controlled conditions (Marineland park, Antibes, France).

Conclusions

Using a set of complementary alpha- and beta-diversity indices, we show that *(i)* cetacean skin microbiome is distinct from the surrounding planktonic communities, *(ii)* the 2 cetacean species host different skin microbiomes, and that *(iii)* inter-individual variability of the skin microbiome was higher than intra-individual variability (*i.e.* between body parts), within each species. The predominant microbial clades on both species were *Gamma*- and *Alpha-proteobacteria*, *Actinobacteria* and *Firmicutes.* However, *Firmicutes*, and especially *Staphylococci*, were six times more abundant on dolphin skin than on killer whale skin.

Overall, the skin microbiome of those two Odontoceti cetaceans was more similar to that of the Mysticeti humpback whale than to microbiomes of fishes or terrestrial mammals.

FEMS7-0555
Environmental Microbiology/Microbial Ecology /Microbial Communities

SOIL- AND PLANT-ASSOCIATED MICROBIAL COMMUNITIES AS DRIVERS FOR THE DECOMPOSITION OF MAIZE RESIDUES IN AN AGRICULTURAL SOIL

A. Chiba[1,2], *M. Schloter*[2], *S. Schulz*[2], *Y. Uchida*[1]
[1]*Hokkaido University, Graduate School of Agriculture, Sapporo, Japan*
[2]*Research Unit Comparative Microbiome Analysis, Department of Environmental Sciences, Neuherberg, Germany*

Backgrounds

Decomposition of plant residues is an important process in agricultural soils. The degradation process is strongly driven by microbes colonizing on plant surfaces as well as soil microbes. However, the interaction among microbiomes derived from soil and plants in relation to residue decomposition is not clearly understood.

Objectives

We evaluated the role of microbes from soil and plants during the degradation process of maize leaves by manipulating their diversities. We postulated that initial degradation processes are unaffected by the diversity of soil and plant microbiomes, however at later stages of litter degradation manipulated diversity will strongly impact the degradation process.

Methods

We prepared three soils, namely “original soil”, “inoculated soil” and “autoclaved soil”. The inoculated soil was an autoclaved soil with microbial cells extracted from the original soil, aiming to manipulate soil microbial diversities. We incorporated litter bags with fresh/sterile maize leaves into these soils. During 6 weeks incubation, the weight of the litter bags was measured. Soil microbial DNA was extracted from the soils and the litter, used for 16S rRNA gene quantification and community analyses.

Conclusions

As expected, the reduction of soil microbial diversity led to a breakdown of the litter degradation. This effect was more severe if sterile litter was added to soils with reduced diversity. In contrast, the degradation rate of sterile litter material applied to non-treated soils was comparable to systems where neither litter nor soils had been sterilized. In summary, the reduction of soil diversity reduced litter degradation rates, independent from the type of litter material.

FEMS7-0021
Environmental Microbiology/Microbial Ecology /Microbial Communities

BIODEGRADATION OF 2,4,6-TRINITROTOLUENE BY CITROBACTER SP. YC4 AND EVALUATION OF ITS CYTO-TOXICOLOGICAL EFFECTS

C.C. Chien[1], S.F. Wei[1], C. Ma[1]
[1]Yuan Ze University, Graduate School of Biotechnology and Bioengineering, Taoyuan, Taiwan R.O.C.

Backgrounds

The compound 2,4,6-trinitrotoluene (TNT) is a secondary explosive widely used for both military and civil purposes around the world. TNT is highly toxic and carcinogenic; therefore, the control of TNT contamination and its remediation is a critical environmental issue. Numerous studies have reported different remediation techniques for TNT-contaminated environments, including natural attenuation, physical or chemical processes, and biological remediation. Among them, bioremediation of TNT by microbial degradation is a very promising technique.

Objectives

The biodegradation products of TNT were evaluated for their cytotoxicity effects. *Citrobacter* sp. YC4 is able to grow in the medium with TNT as sole source of nitrogen. Resting cells and cell extracts of strain YC4 were used to study their ability for TNT biodegradation.

Methods

Cells of strain YC4 were grown in different media and examined for TNT degradation. The degradation products were evaluated for their cytotoxicity effects by WST-1-based cell cytotoxicity assay and comet assay.

Conclusions

Cells grown in the TNT media as sole nitrogen source was able to rapidly transform the compound, in contrast to cells grown in LB medium. The concentration of TNT was decreased from 100 ppm to 0 ppm within 10 hours in the solution containing TNT mixed with TNT-grown cells. Cytotoxicity of solution containing TNT was also decreased after incubated with TNT-grown cells.

FEMS7-0401
Environmental Microbiology/Microbial Ecology /Microbial Communities

IDENTIFICATION AND CLONING OF ECTOINE BIOSYNTHESIS GENES OF AN INDIGENOUS MODERATE HALOPHILE BACTERIUM HALOMONAS SP. ISOLATE

C.C. Chien[1], *C.K. Chang*[1]
[1]*Yuan Ze University, Graduate School of Biotechnology and Bioengineering, Taoyuan, Taiwan R.O.C.*

Backgrounds

Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid), an osmotic pressure compatible solute which serves as a protective substance helping organisms to counteract the external osmotic pressure, is one of the most commonly found osmolytes in nature. Ectoine-producing microorganisms are widely distributed in hyper-saline environments. Ectoine is currently used in biotechnology and cosmetics such as active ingredient in skin care and sun protection products.

Objectives

The aim of the present study was to isolate and characterize ectoine producing microorganisms from marine environment. The genes involved in the ectoine biosynthesis pathway were also studied.

Methods

Indigenous moderate halophile bacterium was isolated from marine sediments. Ectoine production by the isolate was evaluated with another three authentic *Halomonas* spp. (*Halomonas salina* BCRC 17875^T, *Halomonas variabilis* BCRC 17786^T and *Halomonas venusta* BCRC 17787^T) obtained from culture collection center as reference strains. The *ectABC* gene cluster was amplified by PCR for subsequently cloning and expression.

Conclusions

Halomonas sp. MT was isolated and identified according to its 16S rRNA gene sequence determination. The bacterium was able to produce significant amount of ectoine in the presence of osmotic pressure. Genes involved in ectoine biosynthesis (i.e. *ectABC*) in *Halomonas* sp. MT were amplified by PCR. The amplicon of entire *ectABC* gene cluster (ca. 3 kb) was cloned and sequenced. Heterologous expression of these proteins will be carried out in *Escherichia coli*. Salt tolerance and ectoine production by the recombinant *E. coli* will also be examined in this study.

FEMS7-0646
Environmental Microbiology/Microbial Ecology /Microbial Communities

ANALYSIS OF DORMANT CELL DYNAMICS WITHIN PSEUDOMONAS AERUGINOSA BIOFILM

K. Chihara[1], *S. Matsumoto*[2], *S. Miyagawa*[1], *S. Tsuneda*[1]
[1]*Waseda University, Life Science and Medical Bioscience, Shinjuku-ku, Japan*
[2]*Nagoya University, Medical, Nagoya, Japan*

Backgrounds

Existence of dormant cells within biofilms has been indicated as one of factors in chronic infections. Dormant cell, which suppresses cell division and is tolerant to antibiotics, is phenotypic variant of growing cell. To better understand dormant cell dynamics within biofilms, we constructed 3D Individual-based modeling biofilm simulator and predicted dormant cell formation against environmental stresses such as nutrient or oxygen depletion.

Objectives

In this study, a novel dormant cell detection system of *Pseudomonas aeruginosa* PAO1 was constructed. Our objective is to elucidate dormant cell dynamics within growing biofilms by comparing experimental results with simulation results.

Methods

To distinguish dormant cells from growing cells, CFP and YFP were fused at bacterial cytoskeleton FtsZ in PAO1 because FtsZ polymerizes at the middle of the cell during division. Using the constructed dormant cell marker strain, colony biofilm was formed and dormant cells localization was characterized with or without antibiotics.

Conclusions

When the biofilm thickness was under 30 μm, dormant cells localized at the top of the colony biofilm. In contrast, when that thickness was over 30 μm, they localized at the bottom of the colony biofilm. Expectedly, dormant cells survived within biofilm in spite of treatment by ofloxacin. These results suggest that dormant cell localization shifts depending on biofilm thickness with different nutrient and oxygen concentration profiles. We expected dividing cells at the bottom of early biofilm consume nutrient, and thus cells at the top which experienced nutrient depletion turned into dormant state, whereas dormant cells at the bottom of mature biofilm experienced oxygen limitation.

FEMS7-0204
Environmental Microbiology/Microbial Ecology /Microbial Communities

COMMUNAL METABOLISM OF METHANE AND THE RARE EARTH ELEMENT SWITCH

L. Chistoserdova[1], Z. Yu[2], M. Hernandez[3], S. Krause[2], J. Matsen[2], D. Beck[2], M. Lidstrom[2]
[1], SEATTLE, USA
[2]University of Washington, Chemical Engineering, Seattle, USA
[3]Institute of Ecology, Ecology, Xalapa, Mexico

Backgrounds

Metabolism of methane, a major contributor to climate change, is an important part of biogeochemical cycling of carbon. Methanotrophs consume methane for energy and carbon. While methanotrophy has been studied for the past hundred years as a metabolic feature of individual pure cultures, a concept of communal function in methanotrophy has recently emerged.

Objectives

We are investigating the mechanistic details of how and why methanotrophs share carbon with other species, and whether and what they gain in return.

Methods

We manipulated complex natural communities from lake sediment using methane as a sole source of carbon, to determine species persisting in methane-consuming communities (the top-down approach). We built synthetic communities of pure cultures of methanotrophs and non-methanotrophs and tested their behavior under a variety of conditions (the bottom-up approach). We sequenced multiple (meta)genomes and (meta)transcriptomes to gain insights into the genomic potentials and gene expression patterns.

Conclusions

Through microcosm manipulation and metagenomics we identified as key species active in methane consumption bacteria of the family *Methylococcaceae*. As the primary and most abundant satellite types we identified non-methanotrophic methylotrophs of the family *Methylophilaceae*. Two other persistent types were identified as members of Burkholderiales and Flavobacteriales. Through manipulation of synthetic communities followed by metatranscriptomics we identified at least one metabolic node for community cross-talk, two alternative methanol dehydrogenases, one requiring calcium, another requiring rare earth elements (REE), one of the first demonstrations of a biological function for REE. Methanol is thus a carbon compound the methanotrophs share with other community members, regulated by the REE switch.

FEMS7-2704
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROALGAL DIVERSITY FOSTERS STABLE BIOMASS PRODUCTIVITY IN OPEN PONDS TREATING WASTEWATER

D.H. Cho[1], R. Ramanan[2], H.S. Kim[1]
[1]Korea Research Institute of Bioscience and 6 Biotechnology KRIBB, Cell Factory Research Center, Daejeon, Republic of Korea
[2]School of Earth Science Systems- Central 21 University of Kerala, Department of Environmental Science, Kasaragod District, India

Backgrounds

It is established that biodiversity determines productivity of natural ecosystems globally. We have proved that abiotic factors influenced biomass productivity in artificial ecosystems i.e. high rate algal ponds (HRAPs), previously.

Objectives

The present study demonstrates that biotic factors, particularly microalgal diversity, play an essential role in maintaining stable biomass productivity in HRAP treating municipal wastewater by mutualistic adaptation to environmental factors.

Methods

Microalgal diversity, wastewater characteristics, treatment efficiency and several environmental and meterological factors was documented for two years. Multivariate statistical analyses reveal that microalgae in uncontrolled HRAPs adapt to adverse environmental conditions by fostering diversity.

Conclusions

Subsequently, five dominant microalgal strains by biovolume were isolated, enriched, and optimum conditions for high biomass productivity were ascertained. These laboratory experiments revealed that different microalgal strains dominate in different conditions and a consortium of these diverse taxa help in sustaining the algae community from environmental and predatory pressures. Diversity, niche or seasonal partitioning and mutualistic growth are pertinent in microalgal cultivation or wastewater treatment. Therefore, enrichment of selective species would deprive the collective adaptive ability of the consortium and encourage system vulnerability especially in wastewater treatment.

FEMS7-2708
Environmental Microbiology/Microbial Ecology /Microbial Communities

COMPARATIVE GENOME ANALYSIS OF SUBTERCOLA BOREUS, AN ACTINOBACTERIUM RETRIEVED FROM ANTARCTIC ROCKS, SOIL AND FRESHWATER

C. Yong-Joon[1], C. Ahnna[1], K. Ok-Sun[1], L. Jong-Ik[1]
[1]Korea Polar Research Institute, Unit of Antarctic K-route Expedition, Incheon, Republic of Korea

Backgrounds

The actinobacterium Subtercola boreus is psychrophilic species capable of growth at temperatures down to -2 ºC with an optimum at 15-17 ºC. This strain was first discovered in permanently cold groundwater but it was also found in several places at the Antarctica including rocks, terrestrial soil and freshwater.

Objectives

Here, we report the fully assembled genome sequence of S. boreus K300 to find out how this psychrophile can adapt to extreme environments. And the genomic structure and gene content of the type and 5 strains of this psychrophilic bacteria isolated from various Antarctic environments were analyzed and compared.

Methods

The type strain genome sequencing was completed by using hybrid assembly strategy with PacBio P6-C4 20 kb library and Illumina 250 bp paired-end library. Other strains were sequenced by Illumina technology only.

Conclusions

The genome sequence of the S. boreus type strain and the comparative analyses with other Subtercola strains provided a better understanding of psychrophilic adaptation and growth within different environments in this genus.

FEMS7-1611
Environmental Microbiology/Microbial Ecology /Microbial Communities

COMPLETE GENOME SEQUENCE OF SPHINGOBACTERIAL STRAIN SH-48, A PROTEORHODOPSIN-CONTAINING BACTERIUM ISOLATED FROM FRESHWATER IN KOREA

A. Choi[1], K. Baek[2], E.J. Chung[1], G.G. Choi[1]
[1]Nakdonggang National Institute of Biological Resources, Freshwater Bioresources Cullture Research, Sangju-si, Republic of Korea
[2]Nakdonggang National Institute of Biological Resources, Freshwater Bioresources Research, Sangju-si, Republic of Korea

Backgrounds

Proteorhodopsin (PR) was first discovered 10 years ago in uncultivated marine gammaproteobacteria of SAR 86 group. PR is a retinal-binding membrane protein belong to the largest family of microbial rhodopsins. PR is widely distributed in the oceanic environment and freshwater, but no bacteria with PRs have been isolated from freshwater so far.

Objectives

The main aim of the research was to analyze the first complete genome sequence of SH-48.

Methods

Genomic DNA was extracted using a DNeasy blood and tissue kit. Genome sequencing was performed using the PacBio RS II SMRT sequencing technology with a 20-kb insert SMRTbell library constructed. The bacterial genome was assembled *de novo* into one contig, with an average genome coverage of 114.78×, using the PacBio SMRT Portal (2.3.0) and the hierarchical genome assembly process. The data was then submitted to the Rapid Annotation using Subsystem Technology (RAST) server and the National Center for Biotechnology Information genome sequence database. Potential coding sequences were searched for using the Basic Local Alignment Search Tool (BLAST) against the Pfam and COG databases.

Conclusions

Strain SH-48 was isolated from the freshwater stream in Korea using dilution-to-extinction culturing. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain SH-48 formed a distinct phyletic clade and was most closely related to species of the genus *Mucilaginibacter* (81.4 to 93.7%) of the Sphingobacteriia among the validly published species. Here, we present the first complete genome sequence of this strain, which consists of 5,650,162 bp with 35.4 GC content. The genome of strain SH-48 encodes many hydrolytic enzymes for utilizing mainly polysaccharides. The genome also revealed the presence of a proteorhodopsin-encoding sequence together with its retinal-producing pathway. This research provides a genetic basis for better understanding of Sphingobacteriia, as well as insights into the strategies adapted by a rhodopsin-containing photoheterotroph to thrive in the freshwater environment.

FEMS7-2594
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE ASSOCIATION BETWEEN GUT MICROBIOME AND GENE EXPRESSION ON MENOPAUSE MOUSE MODEL

S. Choi[1], S.K. Shin[1], H. Yi[1,2]
[1]Korea University, BK21PLUS Program in Embodiment: Health-Society Interaction. Department of Public Health Sciences. Graduate School, Seoul, Republic of Korea
[2]Korea University, School of Biosystem and Biomedical Science, Seoul, Republic of Korea

Backgrounds

In the period of menopausal transition, the imbalance of estrogen causes obesity. The decreased energy expenditure in postmenopausal period may be linked with efficient energy-generating bacterial strains caused by the low level estrogen environment, but has not been examined.

Objectives

We aimed to compare the composition of intestinal bacteria and the accompanying change of host gene expression during menopausal transition.

Methods

Mouse with ovariectomy-operated(OVX) and sham-operated(SHAM) were prepared and the half of them were provided with high-fat diet. The microbiome in cecum and large intestine was analyzed using 16S rRNA gene sequencing and the gene expression of liver was measured using microarray. The correlation between bacterial abundance and gene expressions was calculated and statistically significant pairs were selected to create a network.

Conclusions

The results demonstrated that estrogen-level plays important roles in microbiota maintenance. The gut microbiome profile of ovariectomy mouse was very similar to control mouse with high-fat diet. The correlation analysis between 652 differentially expressed genes and 137 abundant bacterial species revealed 1160 statistically significant inter-kingdom interactions. The detailed meaning of the individual link in the network is under analyses to determine the metabolic pathway critically determined by the presence or absence of specific bacterial taxa. This study supported by the Cooperative Research Program for Agricultural Science & Technology Development (Project No. PJ010975) and by the National Research Council of Science & Technology (NST) grant (No. CRC-16-01-KRICT) by the Korean government.

FEMS7-0174
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECT OF DIETARY PROCESSED-SULFUR ON GROWTH PERFORMANCE AND GUT MICROBIOTA IN CHICKENS

Y.J. Choi[1], S.S. Lee[1], C.D. Jeong[1], S.K. Kim[2], K.J. Yi[2], J.H. Kim[2]
[1]Sunchon National University, Animal Science and Technology, Suncheon, Republic of Korea
[2]Konkuk University, Department of Animal Science and Technology, Seoul, Republic of Korea

Backgrounds

Sulfur is essential for all animals because S-containing compounds, such as amino acids, hormones, B-vitamins, and co-enzymes, are required for normal metabolic, structural, and regulatory functions of all living organisms. Sulfur compound is highly toxic when directly ingested and causes side effects and thus often physical or chemical methods are used to eliminate harmful ingredients. Nevertheless, insufficient elimination of toxins leads to diseases in animals and thus special care is required.

Objectives

There is limited information about the effect of processed sulfur supplementation on the growth performance, especially correlation of gut microbiota. This study was carried out to investigate the dietary processed sulfur supplementation on the growth performance and gut microbial community in broiler.

Methods

A total of 180 day old Ross broiler chicken were used to investigate the effects of processed sulfur on growth performance and gut microbial community. The two dietary treatments, each with 3 replicates were basal diet and base diet plus the 0.2% (500 ppm) processed sulfur. Bacterial communities in the different regions of gastrointestinal tract of broiler chickens were analyzed by pyrosequencing approach to understand microbial composition and diversity. The DNA samples extracted from 4 different regions along the GIT were subjected to bacterial community analysis by pyrosequencing of the V1-V3 region of 16S rRNA gene.

Conclusions

Providing of 500 ppm sulfur decreased mortality rate and increased feed intake in finisher stage, resulting in weight gain and improving feed conversion rate to positively affect productivity enhancement. At the phylum level, *Firmicutes* was predominant in crop, gizzard and colon with control and treatment. Processed sulfur diet showed lower microbial diversity and quantity in GIT excluding crop.

FEMS7-0950
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECTS OF PROBIOTICS ADDITION IN FATTENING PIGS FEED ON ODOR REDUCTION AND MICROBIAL COMMUNITY

Y.J. Choi[1], A. Biswas[1], S.S. Lee[1], C.D. Jeong[1], M.R. Lee[1]
[1]Sunchon National University, Animal Science and Technology, Suncheon, Republic of Korea

Backgrounds

Probiotics that have been widely marketed in animal industry and used as a replacer for antibiotic growth promoter It is known that probiotic are effective as an environment improvement alternative

Objectives

This study was conducted to know the effect of *Pediococcus acidilactici*, *Bacillus amyloliquefaciens*, and dry yeast on odor reduction potentiality and microbial community of fattening pig

Methods

Fermentation was carried out for 5 days at 37° C by solid-state fermentation method after inoculating each 5% of *Pediococcus acidilactici* DSM 20284^T (3.1x10^8 cfu/mL), *Bacillus amyloliquefaciens sub. plantarum* F2842^T (2.9x10^8 cfu/mL) and dried yeast (1.0x10^8 cfu/g) in sterilized wheat bran. A total of 176 fattening period pigs were used to investigate the effects of probiotic on odor reduction and microbial community. The two dietary treatments, one was control and other treatment was 0.2% fermented wheat bran with control. A total of 38 specimens, including Hydrogen Sulfide, ammonia, Volatile fatty acid (VFA), Indole and Skatole, were analyzed by HPLC and GC/MS at 30 days and bacterial community analysis were done by pyrosequencing

Conclusions

Results of this study revealed that the amount of Hydrogen Sulfide was 1,509 ppb in treatment and 1,950 ppb in control after 30th day. The amount of methyl mercaptan and Skatole at 30th day in treatment group were 5.85 and 0.06 ppb, respectively, compared to 9.05 and 0.08 ppb in the control group, respectively. At the phylum level Firmicutes was predominant in control and treatment. However, abundance of Firmicutes was higher in treatment than control. On the other hand, microbial diversity was higher in control than treatment.

FEMS7-0441
Environmental Microbiology/Microbial Ecology /Microbial Communities

SEASONAL EFFECT IN ABUNDANCE AND PROPERTIES OF PISTACIA TEREBINTHUS-ASSOCIATED COMMUNITIES FROM DRY AREAS

I. Cholakova[1], S. Thijs[1], E. Paredis[1], N. Weyens[1], L. Nacheva[2], A. Vassilev[3], J. Vangronsveld[1]
[1]University of Hasselt, Centre for Environmental Sciences, Diepenbeek, Belgium
[2]Fruit Growing Institute, Department of Propagation- Orchard Management and Biotechnology, Plovdiv, Bulgaria
[3]Agricultural University of Plovdiv, Department of Plant Physiology and Biochemistry, Plovdiv, Bulgaria

Backgrounds

Rhizosphere soil bacteria are beneficial for plants in an environment exposed to abiotic stress such as water shortage. Plant-associated bacteria can promote plant growth and assist their host to cope with osmotic pressures. However, environmental conditions might affect bacterial communities in their composition and abundance, and this may change their functional potential. In this context, it would be interesting to unravel the influence of seasons on the rhizosphere bacterial communities of trees in arid regions.

Objectives

The aim of this study was to investigate whether the abundance and functional properties of rhizosphere bacteria from *Pistacia* trees differ between a more humid spring season and a dry autumn season.

Methods

Soil samples from three different sites in an arid region of Bulgaria were collected to define soil properties and bacterial community composition. *Pistacia terebinthus*-associated cultivable bacteria were isolated from rhizosphere soil samples in two seasons – spring and autumn 2014. Besides the cultivable fraction, isolation of total DNA was performed from soil samples and used for Automated Ribosomal Intergenic Spacer Analysis (ARISA) and shotgun metagenomics sequencing.

Conclusions

Overall, in spring and autumn, high numbers of plant growth promoting (PGP)-isolates colonize the rhizosphere of *Pistacia*, which can help the tree to withstand the harsh conditions. The dominance of certain 16S phylotypes was found to differ between the soils. The functional properties of the microbial communities will be compared between the two seasons using shotgun metagenomics data analyses.

FEMS7-1217
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECT OF EARLY-LIFE FORAGING IN DEVELOPMENT OF PIGLETS

R. Choudhury[1], A. Middelkoop[2], W.J.J. Gerrits[3], B. Kemp[2], J.E. Bolhuis[2], M. Kleerebezem[1]
[1]Wageningen University & Research, Host Microbe Interactomics- Animal Sciences, Wageningen, The Netherlands
[2]Wageningen University & Research, Adaptation Physiology Group- Animal Sciences, Wageningen, The Netherlands
[3]Wageningen University & Research, Animal Nutrition Group- Animal Sciences, Wageningen, The Netherlands

Backgrounds

Early-life and weaning are associated with dynamic changes of the gut microbiome in pigs. Early-life microbiome development is driven by exogenous (e.g., diet and environment) and endogenous (host derived) factors, and importantly modulates host physiology, which can persist throughout life. In nature, piglets start to forage with the sow already a few days after birth.

Objectives

Here, we aim to determine the effect of early-life foraging (pre-weaning access to solid feed) on growth, development and behaviour of piglets, their gut microbiome composition, and intestinal physiology.

Methods

Piglets were followed from birth until 6 weeks of age, and various samples (rectal swabs, blood, saliva, etc.) were collected at multiple time points. Next to the sow's milk, the treatment group was provided with a customised fibre-rich early-foraging diet from 2 days after birth, whereas the control group was only drinking sow milk. Physiological and behavioural measurements were collected during the pre- and post-weaning period. During the same period rectal swabs were collected to investigate community structure of the intestinal microbiome by 16S rRNA gene analysis. Moreover, a subset of piglets was sacrificed at weaning (at 28 days of age) and intestinal samples were collected, allowing the differential analysis of histological and molecular parameters in the experimental versus the control group.

Conclusions

By integrative data analysis, the study intends to provide insight into the relationship of early-foraging with pigs' welfare, health, and development. This knowledge can lead to sustainable husbandry regimes that mimic natural conditions for the production of healthier and more resilient pigs.

FEMS7-1437
Environmental Microbiology/Microbial Ecology /Microbial Communities

MARINE MICROBIAL INTERACTIONS SHOWCASED THROUGH EXOPROTEOMES

J.A. Christie-Oleza[1]
[1]*University of Warwick, School of Life Sciences, Coventry, United Kingdom*

Backgrounds

Microbial phototroph-heterotroph interactions propel the engine that results in the biogeochemical cycling of individual elements and, hence, studying the molecular basis of these key interactions is crucial for the understanding of marine systems. Cellular proteomics is a reliable tool to analyse how living organisms adapt to varying conditions, but the extensive datasets that are generated are swamped by processes from the central metabolism that occur independently to the extracellular environment.

Objectives

Here I present the advantages of analysing the extracellular proteome for studying the molecular processes that take place during microbial interactions and how proteins targeting specific cellular compartments can sometimes be found in the extracellular milieu.

Methods

High-throughput proteomics was used to analyse the exoproteome of an extensive array of co-cultured marine heterotrophic and phototrophic organisms.

Conclusions

The secreted fraction of the encoded proteome had a much higher incidence of lineage-specific proteins than the cytosolic fraction and exported proteins are largely uncharacterized to date compared to proteins from the cytosolic fraction. This suggests that the genomic and functional diversity of these organisms lies largely in the diverse pool of novel functions these organisms export to/through their membranes playing a key role in community diversification, e.g. for niche partitioning or evading predation. Furthermore, exoproteomes show i) a large number of transport systems giving information on the nutrients that are generated/targeted by each microbe within the co-culture, ii) an interesting array of strain-specific exoproteins involved in motility, gene exchange and mutualistic or hostile interactions, and iii) uncharacterised exoenzymes that require further characterisation.

FEMS7-0052
Environmental Microbiology/Microbial Ecology /Microbial Communities

DETERMINATION OF CR(VI) IN WASTEWATER USING MICROBIAL FUEL CELL-BASED BIOSENSOR OPERATED WITH EXIGUOBACTERIUM SP. YC210 AT THE ANODE

C.Y. Chen[1], M.C. Hsieh[2], S.T. Lin[2], Y.C. Chung[2]
[1]Hsing Wu University, Department of Tourism and Leisure, New Taipei city, Taiwan R.O.C.
[2]China University of Science and Technology, Department of Biological Science and Technology, Taipei city, Taiwan R.O.C.

Backgrounds

Cr(VI) is extensively used in electroplating, resistant alloys and leather tanneries. It is a toxic, carcinogenic and mutagenic chemical. The currently analytical methods for the Cr(VI) determination include atomic absorption spectroscopy, inductively coupled plasma mass spectroscopy, ion chromatography, and their combination with chromatographic techniques. These techniques exhibit high accuracy and selectivity, but they are usually tedious and expensive.

Objectives

In this study, we would like to develop a biological method to determine Cr(VI) in wastewater. A facultatively anaerobic, halotolerant, and moderately thermophilic bacterium, *Exiguobacterium* sp. YC210 was isolated and inoculated in a microbial fuel cell (MFC) to evaluate its feasibility as a biosensor or an early warning device for Cr(VI) determination.

Methods

Effects of coexisting cation ions (Zn^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, Na^{+}, Cr^{3+}), anion ions (Cl^{-}, $SO_4^{=}$) and pH values on Cr(VI) determination using MFC-type biosensor were evaluated. Also, Cr(VI) concentrations in five types of real wastewaters were measured by the biosensor and through standard colorimetric method.

Conclusions

Two regression equations for the Cr(VI) concentration and voltage output of the MFC-type biosensor were established. One is determined to be $y = -19.6\,x + 397.2$ with Cr(VI) concentrations ranged from 0 to 4 mg/L. The other equation was determined to be $y = -10.7\,x + 155.5$ with Cr(VI) concentration ranged from 4 to 20 mg/L. Effects of coexisting cation ions (< 45 mg/L), Cl^{-} (< 15 mg/L), and $SO_4^{=}$ (< 20 mg/L) on the performance of MFC-type biosensor were insignificant (p>0.05). The *Exiguobacterium* sp. biosensor for Cr(VI) determination could be fallen into a wide range of pH values (4-10). Compared with Cr(VI) concentrations measured through colorimetric method, the designed biosensor measurements in wastewaters were accurate and had low deviations (-1.8% to 3.2%). Thus, the developed MFC-type biosensor has the potential to be used as an early warning device to protect ecosystems.

FEMS7-0545
Environmental Microbiology/Microbial Ecology /Microbial Communities

METAGENOMIC ANALYSIS OF SOIL BACTERIA RESILIENCE IN A NEMATICIDE TREATED SOIL

M. Colagiero[1], L.C. Rosso[1], I. Pentimone[1], S. Convertini[2], A. Ciancio[1]
[1]CNR - Istituto per la protezione sostenibile delle piante, DISBA, Bari, Italy
[2]Reagri srl, -, Massafra Taranto, Italy

Backgrounds

Several agricultural practices affect soil bacterial diversity. Few data are available on the effects of chemical treatments such as nematicides.

Objectives

We aimed at studying the changes induced on soil bacteria by nematicides, through metagenomic analyses.

Methods

Six treatments were applied on greenhouse tomato infested by the nematode *Meloidogyne incognita* (Carovigno, Italy). They included 1,3-dichloropropene (1,3-D at 150, 200 or 250 l/ha), fenamifos (42 l/ha) and DMDS (300 kg/ha), with untreated parcels as controls. Soil (three replicates) was sampled at transplant and one month later. RNA was extracted and retrotranscribed, sequencing the hypervariable V3-V4 regions of the 16S rRNA gene on a MiSeq SystemÔ (Illumina). Contigs were assembled with PandaSeq and analyses performed with Qiime and STAMP.

Conclusions

A total of 4405 OTUs was identified in the first sampling. The alpha-diversity did not significantly differ among samples, with the exception of fenamifos-treated soil with a lower chao-1 index. Proteobacteria, Actinobacteria, Plancomycetes, Chloroflexi and Acidobacteria were mostly represented, with classes: Alphaproteobacteria, Actinobacteria, Phycisphaerae, Solibacteres and Acidimicrobiia. The predominant order was Rickettsiales, with Actinomycetales, Rhodospirillales, Sphingomonadales and Rhizobiales. Principal coordinate analysis showed samples were aggregated, apart of two controls. 4113 OTUs were found in the second sampling. Alpha-diversity was lower for 1,3-D at 150 and 200 l/ha than in control, but not significantly different at highest dose. Chao-1 index, accounting for rare taxa, was significantly higher in samples treated with 1,3-D at 200 l/ha. Data suggest that nematicides did not reduce the diversity of soil bacteria, which showed a resilient response.

FEMS7-0453
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROALGAL PREMATURE SENESCENCE PROVOKED BY CHEMICAL STRESS

M. Esperanza[1], M. Seoane[1], C. Rioboo[1], C. Herrero[1], Á. Cid[1]
[1]Universidade da Coruña, Bioloxía Celular e Molecular, A Coruña, Spain

Backgrounds

Organisms live in a constant struggle to cope with damaging agents. The inevitable accumulation of damage leads to the deterioration of cell components and functions. When damage accumulates irreversibly, cells can permanently arrest the cell cycle or trigger cell death programs. Furthermore, heterogeneity within the environment leads to nutrient patchiness and nutrients become depleted leading to cell death. This phenomenon is analogous to cultures grown in closed systems under laboratory conditions.

Objectives

Potential senescence of *Chlamydomonas reinhardtii* cells, as a response to a sublethal concentration of the herbicide atrazine, was studied.

Methods

Several senescence-related parameters were monitored: viability, reactive oxygen species (ROS) production, caspase activity, morphology of nuclei and the presence of auto-phagosomes. RNA-Seq technique was applied to obtain detailed quantitative transcriptomic profiling of algal cells exposed or not to atrazine, looking for similarities in the regulation of transcription.

Conclusions

Cell viability remained above the 96% for all conditions, ROS levels increased in all cultures with respect to 24 h-control, as well as caspase activity, cells with auto-phagosomes and alterations in the nuclei morphology. RNA-Seq technique displayed 27, with well-known function, differentially expressed genes between control cultures at 96 h and atrazine exposed cultures at 24 h. Many cellular processes were affected, but the most significant changes were observed in genes implicated in cell cycle. Results indicate that the production of ROS caused by exposure to atrazine during 24 h induces oxidative damage, possibly leading to the observed premature senescence in microalgal cells.

CGL2010-15993/BOS and Xunta de Galicia predoctoral grants (M.E. & M.S.)

FEMS7-0455
Environmental Microbiology/Microbial Ecology /Microbial Communities

ACUTE TOXICITY OF OMEPRAZOLE ON THE MICROALGA TETRASELMIS SUECICA

M. Seoane[1], M. Esperanza[1], C. Rioboo[1], C. Herrero[1], Á. Cid[1]
[1]Universidade da Coruña, Bioloxía Celular e Molecular, A Coruña, Spain

Backgrounds

Omeprazole (OMP) is one of the most commonly used drugs worldwide. It belongs to a group of pharmaceuticals called proton pump inhibitors that act by irreversibly blocking the terminal stage of gastric acid secretion, inhibiting the gastric proton pump (H+/K+ATPase). Therefore, OMP decreases the amount of acid produced in the stomach and it is used for the treatment of gastro-intestinal disorders. Although it is daily consumed in high quantities and is commonly detected in waters worldwide, it is a poorly studied compound and relatively little is known about its ecotoxicity.

Objectives

The aim of this study was to evaluate the potential acute toxicity of increasing concentrations of OMP (1.6 to 2.8 ng cell^{-1}) on the marine microalga *Tetraselmis suecica.*

Methods

Several cytotoxicity biomarkers were analysed by flow cytometry after 24 h of exposure, completing a 12:12 h light:dark cycle.

Conclusions

Results showed that OMP caused a decrease in growth and autofluorescence, swelling of the cells, intracellular acidification and hyperpolarization of cytoplasmic and mitochondrial membranes. In addition, large amounts of reactive oxygen species (ROS) were generated which resulted in a decrease in the percentage of the viable population. However, the viable population showed an increase in the metabolic activity as a response to the stress. In conclusion, OMP may trigger receptors in unicellular organisms that regulate the proton flux in the cytosol. It disturbs pH homeostasis and provokes an early accumulation of ROS that results in a rapid cell death in cells cultured with the highest concentration assayed.
Xunta de Galicia fellowship (M.S. & M.E.)

FEMS7-1793
Environmental Microbiology/Microbial Ecology /Microbial Communities

ISOLATION OF PSYCHROTOLERANT YEAST STRAINS WITH POTENTIAL FOR BIODEGRADTION OF PHENOL AND GLYPHOSATE

H. Cieslinski[1], A. Terebieniec[1], N. Filipowicz[1]
[1]Gdansk University of Technology, Molecular Biotechnology and Microbiology, Gdansk, Poland

Backgrounds

Nowadays, at a time of industrial development, the environmental pollution increase rapidly. Part of the pollution is an effect of intensive farming and pesticide overuse. The most common used agrochemical is glyphosate (PMG) - the active ingredient of Roundup, which is consider as possibly carcinogenic.

Another important water and soil pollution, according to its influence on human health, is phenol. The main sources of this hazardous compound are chemical industry and coal conversion processes.

Biological methods of these pollutants treatment base on biodegradation by microorganisms which are able to utilize compounds as a source of carbon needed to growth (for phenol) or to utilize carbon, nitrogen or phosphorus (for PMG).

Objectives

Isolation of psychrotolerant yeast strains from soil and water samples and testing their ability to organic pollutants degradation.

Methods

Microorganisms were isolated form soil and water samples collected from agricultural, industrial or peat-bog areas from Poland. Pure cultures were grown on YPD agar with ampicillin and chloramphenicol in 18^{o}C.

For nitrogen/phosphorus assimilation tests, isolates were cultured in liquid Czapek Dox Medium (CDM) with PMG at 4 mM and 2 mM concentrations as a sole nitrogen and phosphorus source respectively. For carbon assimilation test, isolates were cultivated in MSM medium supplemented with phenol at 500 – 2000 mg/l concentration. All strains were cultivated under 18°C.

Conclusions

The studies revealed that psychrotolerant yeast strains isolated from water or soil samples are excellent candidates for effective biodegradation of phenol and PMG. Isolated strains can be potentially used in waste water or soil treatments in temperate climate.

FEMS7-2245
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE INTERACTION BETWEEN ENDOPHYTES AND SUGARCANE RESULTS IN CHANGES ON NITROGEN METABOLISM AND, RHIZOSPHERIC AND ENDOPHYTIC BACTERIAL COMMUNITIES

R. Freitas-Iório[1], M. Dimitrov[2], M. Cipriano[3], S. Andrade[4], E. Kuramae[2], A. Silveira[3]
[1]Agronomic Institute, Soil and Environmental Resource, Campinas, Brazil
[2]Netherlands Institute of Ecology, Microbial Ecology, Wageningen, The Netherlands
[3]Agronomic Institute, Soils and Environmental Resources, Campinas, Brazil
[4]University of Campinas, Plant Physiology Department, Campinas, Brazil

Backgrounds

Some endophytic bacteria have been used in different crops worldwide as plant-growth promoting bacteria. Nevertheless, the effects of these bacteria in sugarcane plants are not completely clear.

Objectives

The main goal was to evaluate the effect of sugarcane inoculation with six endophytic isolates in regards to the plant nutritional state, some aspects of nitrogen metabolism and its influence on endophytic and rhizospheric bacterial communities.

Methods

The endophytic isolates (*Burkholderia caribensis* IAC/BECa-088, *B. tropical* IAC/BECa-135, *Kosakonia radicitans* IAC/BECa-090 and 095*, Herbaspirillum* sp. IAC/BECa-152 and *Pseudomonas fluorescens* IAC/BECa-141) were inoculated on sugarcane seedlings and the foliar free amino acids levels and polyamine profiles, SHR5, GS1 and GS2 genes transcription, shoot mineral nutrition and, endo and rhizospheric bacterial communities were determined.

Conclusions

Our data revealed that the inoculation of all isolates was able to improve plant growth. The isolates changed the level of foliar free amino acids and polyamine profiles reducing the concentration of citruline, putrecine, glycine, alanine, glutamate, glutamine, proline and aspartate. SHR5 transcript levels in sugarcane leaves were reduced in some treatments, suggesting a successful sugarcane association with the endophytic strain *K. radicitans* IAC/BECa-095 and *P. fluorescens* IAC/BECa-141. All isolates impacted endo and rhizospheric bacterial communities, increasing the abundance of some families such as *Xanthomonadaceae*, *Burkholderiaceae*, *Methylobacteriacceae*, *Rhizobiaceae* and *Microbateriaceae*. The isolate *P. fluorescens* IAC/BECa-141 promoted the highest benefits in terms of plant growth probably due to its effective interaction, through changes in nitrogen metabolism and on bacterial communities at the rhizosphere and endosphere compartments.

FEMS7-1627
Environmental Microbiology/Microbial Ecology /Microbial Communities

OCCUPANCY, VENTILATION AND USE OF INDOOR ENVIRONMENTS DICTATE THE MAKEUP OF THEIR MICROBIAL INHABITANTS

C. Ge[1], S. Huang[1], M. Canales[1], E. Cloutman-Green[1], L. Ciric[1]
[1]UCL, Civil- Environmental & Geomatic Engineering, London, United Kingdom

Backgrounds

As we are spending more time indoors, air quality within buildings is becoming a concern. Ventilation is used to maintain thermal comfort and dilute contaminants such as carbon dioxide, particulate matter and biological contaminants including microbes. However, higher occupancy and more air-tight buildings are leading to worse indoor air quality.

Objectives

The main objectives of the study were to: 1) measure levels number of physical and biological contaminants in different spaces in a university building; 2) assess whether levels of contaminants were correlated to occupancy; 3) assess whether microbial contamination is closely correlated to levels of any of the physical contamination parameters; and 4) assess what microbial taxa are present depending on occupancy and activity.

Methods

Four spaces with different ventilation strategies (e.g. natural, mechanical) and which are utilised in different ways (e.g. classroom, lecture theatre, office) were investigated. Repeat measurements of the following parameters were collected: occupancy, temperature, relative humidity, size-resolved particulate matter, carbon dioxide concentration, colony counts on a non-selective agar and colony counts on an agar selecting for common human pathogens. The measures for all parameters were standardised and correlations were investigated using Pearson's correlation coefficient.

Conclusions

Results indicate that total microbial contamination is closely correlated to occupancy and that the presence of different taxa is influenced by the activity taking place in the space as well as the ventilation strategy. Within the mechanically ventilated spaces, a close correlation between colony counts from air and particulate matter sized between 2.5 and 5 μm.

FEMS7-1925
Environmental Microbiology/Microbial Ecology /Microbial Communities

USING METAGENOME BASED GENOME RECONSTRUCTION TO UNDERSTAND BACTERIAL AND ARCHAEAL ASSEMBLAGES IN THE SOUTHERN OCEAN

C. Matthew[1], *S. Vikram*[1], *T. Makhalanyane*[1]
[1]*Center for Microbial Ecology and Genomics- University of Pretoria, Genetics, Pretoria, South Africa*

Backgrounds

Microbial communities (Bacteria and Archaea) are ubiquitous across ecosystems and regulate key biogeochemical cycles. Due, in part, to difficulties related to the cultivation of 99% of microbes in the laboratory, little is known regarding precise mechanisms that allow microbial guilds to fulfill their ecological roles. Culture-independent analyses allow us to bypass this 'cultivation bottle-neck', and generate vast sequence data-sets which offer crucial insights into microbial metabolic potential. The Southern Ocean (SO) accounts for 40% of all oceanic carbon cycling. Evidence suggests that microbes (predominantly Archaea) are significant mediators in cycling of carbon, nitrogen and sulphur through chemolithoautotrophic pathways.

Objectives

To better understand the physiological roles of Bacteria and Archaea, we aim to reconstruct microbial genomes from SO metagenomes

Methods

Shotgun metagenome sequences were generated from samples recovered from the SO. These sequences were trimmed and filtered to remove low quality reads and then assembled using SPAdes. BLASTp was performed on translated contigs to assess functional potential and diversity of the metagenomes. Contigs were binned using composition dependent and independent binning algorithms to attain the best initial bins.

Conclusions

The assembly of the 6 data-sets produced ~38,780 contigs per sample (≥500 bp) which were used to bin 31 bacterial and archaeal genomes. Of these, 13 showed above 40% completeness. The BLASTp analysis revealed dominance of *Proteobacteria* in all 6 data-sets with a higher relative abundance of *Halobacteria* (MG*III* Archaea). Our analysis suggests that contrast to other marine environments where *Cyanobacteria* drive key ecosystem processes, *Proteobacteria* are the major functional guilds in this marine environment

FEMS7-0907
Environmental Microbiology/Microbial Ecology /Microbial Communities

COLD-ACTIVE PROTEASE PRODUCING BACTERIAL STRAINS DERIVED FROM SALMO TRUTTA MACROSTIGMA

D.N. Colak[1], M. Kacagan[2], S. Canakci[2], A.O. Belduz[2]
[1]Giresun University- Vocational School of Dereli, Department of Forestry, Giresun, Turkey
[2]Karadeniz Technical University- Faculty of Science, Department of Biology, Trabzon, Turkey

Backgrounds

Proteolytic enzymes are ubiquitous in occurrence, being found in all living organisms, and are essential for cell growth and differentiation. 60-65% of the industrial enzymes are belong to the protease family and mostly used in detergent industry, leather tannig, waste treatment, pharmacy exc. With the high activity and stability of proteases at low temperatures, the costs of industrial applications could be decrease significantly.

Objectives

In this work, the bacterial flora of *Salmo trutta macrostigma* was screened for cold active protease activity and industrial utility.

Methods

S. trutta macrostigma strains were collected from a cold stream of Balıkırmagi plateau (Duzkoy/Trabzon). The gastric mill and the intestine extract of the fish was cultivated at 37°C for 2 days in LB medium for bacterial growth. Single colonies were selected, isolated and 16S rRNA analysed. Bacterial isolates were incubated in LB medium with 1% of skim milk at 4°C, 8°C, 15°C, 25°C, and 37°C respectively. Protease positive strains were determined. 1% of azocasein was used for further activity assays.

Conclusions

Nine of the strains isolated from fish intestine was protease positive. Five of them belongs to the genus *Flavobacterium* sp., and 4 of them to *Bacillus* sp. All of the strains had exhibited the protease activity at 20-40°C, except for *Flavobacterium* sp. MT3 which showed the otimum protease activity at 8°C.

In this work 9 protease positive bacterial strains were isolated from *Salmo trutta macrostigma*, one of them had cold-active (4-25°C) protease activity. The protese of *Flavobacterium* sp. MT3 will be characterized and analysed for industrial applications.

FEMS7-1896
Environmental Microbiology/Microbial Ecology /Microbial Communities

CRYPTOCOCCUS SPP. PRESENT IN THE LUNG CORE MICROBIOME

M.F. Colom[1], E. Rubio-Portillo[2], D. Orts[3], E. Llorca[3], E. Revelles[1], J. Antón[4]
[1]Universidad Miguel Hernández, Producción Vegetal y Microbiología, Sant Joan d'Alacant, Spain
[2]Universidad de Alicante, Fisiología- Genética y Microbiología, San Vicente del Raspeig, Spain
[3]Hospital General Universitario de Elda, Sección Neumología. Servicio de Medicina Interna, Elda, Spain
[4]University of Alicante, Fisiología- Genética y Microbiología, San Vicente del Raspeig, Spain

Backgrounds

The use of the highly-throughput sequencing-based studies have led to the detection of microorganisms colonizing the lower respiratory tract of healthy individuals. Most of these studies focus on the description of the bacterial community while the fungal microbiome remains basically unexplored.

Objectives

Here, we explore the hypothesis that *Cryptococcus spp* yeasts are present in the human lower airways and they may cause cryptococosis, when there is a decrease in the host defenses or a local microbiome disruption.

Methods

Thirteen Brochioalveolar Lavage samples (BAL) were obtained by bronchoscopy in the General Universitary Hospital in Elda (Alicante. Spain). The samples were processed for DNA extraction (and for cultures and other cellular studies). The fungal Internal Transcribed Spacer (ITS) of the extracted DNA, was amplified by conventional PCR. The product was purified and a second seminested-PCR was performed with primers ITS4-86. The product was sequenced by the *illumina system*. Results of the massive sequencing were analyzed by QIIME 1.8.0 (Quantitative Insights Into Microbial Ecology) and the UNITE (User-friendly Nordic ITS Ectomycorrhiza) database was used for taxonomic assignments of sequences by BLAST.

Conclusions

Cryptococcus spp is a common member in the lower respiratory tract of all samples (100%) with a high prevalence of *C. neoformans* species. BAL samples showed to have more than 50 fungal species belonging to both phyla (*Basidiomycota* and *Ascomycota*) among which *Aspergillus* and *Candida* species were also detected.
This is a first evidence of the prevalence of *Cryptococcus* in the respiratory tract of the normal population.

FEMS7-2561
Environmental Microbiology/Microbial Ecology /Microbial Communities

DETERMINATION OF THE MICROBIAL COMMUNITY IN THE BROILER CHICKEN SOIL

A. Comte[1], *M. Diarra*[2], *T. Dumonceaux*[1], *E. Topp*[3], *J. Town*[1]
[1]*Agriculture-Agri-food Canada, Bioproduct and processing, Saskatoon, Canada*
[2]*Agriculture-Agri-food Canada, Guelph Research and Development Centre, Guelph, Canada*
[3]*Agriculture-Agri-food Canada, London Research and Development Centre, London- Ontario, Canada*

Background:

Enterobacteria are found in large numbers in poultry litter. Some of these bacteria could be potentially pathogenic and/or resistant to antibiotics.

Objectives:

The objective was to study the evolution of bacterial community**,** and to quantify the dominant bacteria in soil fertilised with litter from broiler chickens fed with or without antimicrobial agents.

Methods:

An agricultural soil was amended with litter from 36-day-old broiler chickens fed with diets supplemented with the commonly used antimicrobials monensin, chlorotetracycline, narasin, or virginiamycin. Soil samples were collected during 8 months for genomic DNA extraction, which was used as template for *cpn60* universal target amplification. The amplicons were purified by gel and pooled for sequencing. Operational Taxonomic Unit (OTU) identification was based on watered BLAST results. Abundant OTU and antimicrobial resistance genes (ARG) were quantified in soil samples using droplet digital PCR.

Conclusion:

Soil fertilised with litter from antibiotic treated birds showed decreased diversity over time compared to soil receiving litter from the control birds. Moreover, non-amended soil presented less bacterial diversity compared to litter-amended soils. All litter-amended soils were dominated by a microorganism with a 99.6% *cpn60* nucleotide sequence identity to *Escherichia coli*. Using ddPCR, we have shown that *E.coli and all resistance genes* were abundant in all litter-amended soil samples, particularly in soils amended with litter from virginiamycin-fed birds, and *E.coli* and ARG decreased over time. This study shows that bacterial community of agricultural soils can be changed when poultry litter is used as fertilizer and that such soils can be a reservoir of antibiotic various resistant genes.

FEMS7-3236
Environmental Microbiology/Microbial Ecology /Microbial Communities

DETERMINATION OF THE MICROBIAL COMMUNITY IN SOIL AMENDED WITH THE BROILER CHICKEN

A. Comte[1], M. Diarra[2], T. Dumonceaux[1], E. Topp[3], J. Town[1]
[1]Agriculture-Agri-food Canada, Bioproduct and processing, Saskatoon-SK, Canada
[2]Agriculture-Agri-food Canada, Guelph Research and Development Centre, Guelph-ON, Canada
[3]Agriculture-Agri-food Canada, London Research and Development Centre, London- ON, Canada

Backgrounds

Enterobacteria are found in large numbers in poultry litter. Some of these bacteria could be potentially pathogenic and/or resistant to antibiotics.

Objectives

The objective was to study the evolution of bacterial community**,** and to quantify the dominant bacteria in soil fertilised with litter from broiler chickens fed with or without antimicrobial agents.

Methods

An agricultural soil was amended with litter from 36-day-old broiler chickens fed with diets supplemented with the commonly used antimicrobials monensin, chlorotetracycline, narasin, or virginiamycin. Soil samples were collected during 8 months for genomic DNA extraction, which was used as template for *cpn60* universal target amplification. The amplicons were purified by gel and pooled for sequencing. Operational Taxonomic Unit (OTU) identification was based on watered BLAST results. Abundant OTU and antimicrobial resistance genes (ARG) were quantified in soil samples using droplet digital PCR.

Conclusions

Soil fertilised with litter from antibiotic treated birds showed decreased diversity over time compared to soil receiving litter from the control birds. Moreover, non-amended soil presented less bacterial diversity compared to litter-amended soils. All litter-amended soils were dominated by a microorganism with a 99.6% *cpn60* nucleotide sequence identity to *Escherichia coli*. Using ddPCR, we have shown that *E.coli and all resistance genes* were abundant in all litter-amended soil samples, particularly in soils amended with litter from virginiamycin-fed birds, and *E.coli* and ARG decreased over time.

This study shows that bacterial community of agricultural soils can be changed when poultry litter is used as fertilizer and that such soils can be a reservoir of antibiotic various resistant genes.

FEMS7-1155
Environmental Microbiology/Microbial Ecology /Microbial Communities

SOIL MICROBIOTA RESPIRATION ANALYSIS USING NEW OXYGEN-SENSITIVE MICROPLATES

J.L. Copa-Patiño[1], Á. Criado Fornelio[1], C. Verdú Expósito[1], T. Martín Pérez[1], C. Moracho Vilrriales[1], I. Heredero Bermejo[1], J. Soliveri de Carranza[1], J. Pérez Serrano[1]
[1]Universidad de Alcalá, Biomedicina y Biotecnología, Alcalá de Henares, Spain

Backgrounds

Usually respiration of soil microorganisms is monitored by measuring CO_2 efflux. Most of the current laboratory techniques employed for such purpose are cumbersome and difficult to adapt to micro-method format.

Objectives

Evaluate a second generation of microplates containing a new fluorophore covalently bound to the bottom of the well for laboratory microorganism respiration studies in pure culture or in soil samples.

Methods

Second-generation oxygen-sensitive plates Oxoprobics® OSMs capable of performing a highly sensitive measurement of oxygen content were evaluated for monitoring respiration in the course of axenic culture of microorganisms. Subsequently, either soil samples or their water extracts were used. However, the water extract procedure was much more convenient and therefore used in further experiments to study the respiration in presence of different chemicals.
Soil respiration curves generally showed no lag phase, starting by an exponential oxygen consumption phase and reaching a plateau after 8-10 h of incubation at 25 °C. In presence of streptomycin and penicillin, no reduction in soil respiration was detected. Kanamycin plus neomycin, thrimetroprim and 5-fluorocytosine exhibited limited inhibitory effects. In contrast, some chemicals like copper sulphate, amphotericin B, Dodine and Fosetyl, noticeably reduced fluorescence readings. Finally, insecticides and soil amendments were neutral or increased respiration.

Conclusions

Oxygen-sensitive microplates are reliable, convenient, and yield quantitative results. Moreover, the system is relatively inexpensive and amenable to automation. However, results obtained using soil water extracts were different from those derived from undisturbed soil aggregates or slurries studied under field conditions.

FEMS7-2424
Environmental Microbiology/Microbial Ecology /Microbial Communities

SPREAD OF VARIABLE ARSENIC TOLERANCE GENES AMONG ENTEROCOCCUS SPP FROM DIFFERENT HOSTS, ENVIRONMENTS AND GEOGRAPHICAL REGIONS (<1906-2015)

J. Mourão[1], A. Rebelo[2,3], A.R. Freitas[1], T.M. Coque[4], A. Almeida[2], L. Peixe[1], P. Antunes[1,5], C. Novais[1]
[1]UCIBIO/REQUIMTE. Faculdade de Farmácia. Universidade do Porto, Ciências Biológicas, Porto, Portugal
[2]LAQV/REQUIMTE. Faculdade de Farmácia. Universidade do Porto, Ciências Químicas, Porto, Portugal
[3]CISA- Centro de Investigação em Saúde e Ambiente. Escola Superior de Saúde- Politécnico do Port o, ATC Saúde Ambiental, Porto, Portugal
[4]Hospital Universitario Ramón y Cajal, Servicio de Microbiología, Madrid, Spain
[5]Faculdade de Ciências da Nutrição e Alimentação. Universidade do Porto, FCNAUP, Porto, Portugal

Backgrounds

Successful bacteria accumulate different genetic features shaping their evolution and fitness to diverse environments/hosts. Arsenic-compounds are widespread in nature and possibly contribute to selection of particular strains.

Objectives

To evaluate the dispersion of *arsA* genes among *Enterococcus* from several origins, geographical regions and temporal timeframes.

Methods

arsA (coding for arsenical-pump-driving-ATPases) were searched in GenBank *Enterococcus* genomes and used to construct a maximum-likelihood phylogenetic-tree. A PCR scheme+sequencing was developed to detect *arsA* alleles from all phylogenetic subgroups identified among 315 isolates (Portugal; human/animal/environment/food; 1996-2012). Na_2HAsO_4 susceptibility was evaluated by agar dilution (0,25 to 128mM; n=102 isolates).

Conclusions

Two major phylogenetic groups (A;B) and 5 subgroups (55-70% nucleotide identity; AI-GenBank-EFU15692.1, AII-EEU88411.1, BI-EOT39237.1, BII-SET88118.1, BIII-EOH82892.1; n=42 isolates) were detected. In all phylogenetic-subgroups *arsA* was distributed in different sources and/or species: *arsA*_AI-n=28 (human/animal/environment/food/feed; *E.faecalis-24/E.faecium-3*; Europe/North-America/Australia/Africa/Asia; <1906-2015); *arsA*_AII-n=13 (human/animal/feed; *E.faecalis*-13*;* Europe/North-America/Australia/Africa/Asia; 1951-2012); *asrA*_BI-n=6 (*E.dispar-1/E.malodoratus-1/E.avium-1/Enterococcus-3*; human/food; North-America; <1991-unknown); *arsA*_BII-n=7 (human; *E.faecium-1*/*E.raffinosus-1/E.devriesei*-1/*Enterococcus*-4; Europe; unknown date); *arsA*_BIII-n=2 (animal; *E.villorum-1/E.hermanniensis-1*; Europe/North-America; 1981-unknown). *arsA*_AI+*arsA*_AII were often associated (n=13). PCR+sequencing identified *arsA*-AI/AII/BII in 8% (n=26/315) of Portuguese *Enterococcus* also from diverse sources and species. Variable Na_2HAsO_4 phenotypes were observed: *arsA*_AI+*arsA*_AII (MIC=32-64mM/n=6); *arsA*_AI (MIC=4-8mM/n=2; 32-128mM/n=4); *arsA*_AII (MIC=0,5-2mM/n=7); *arsA*_BII (MIC=4mM/n=1); no-genes (MIC=0,5-2mM/n=78, MIC=8-128mM/n=4 suggesting the occurrence of new genotypes). *arsA* have been spread in the last 100-years among *Enterococcus* from diverse origins. The A/B phylogenetic groups seem to reflect diverse evolutionary pathways, with A-group including *E.faecalis/E.faecium* and B-group mainly other species. These data potentially reflect diverse genetic exchanges in bacterial communities including *Enterococcus* and other *Firmicutes* also carrying these *arsA* variants.

FEMS7-2444
Environmental Microbiology/Microbial Ecology /Microbial Communities

ARSENIC TOLERANCE GENES ARE FREQUENT AMONG SALMONELLA PIG-ASSOCIATED CLINICALLY-RELEVANT CLONES

J. Mourão[1], A. Rebelo[2,3], J. Machado[4], A. Almeida[2], L. Peixe[1], C. Novais[1], P. Antunes[1,5]
[1]UCIBIO/REQUIMTE. Faculdade de Farmácia. Universidade do Porto, Ciências Biológicas, Porto, Portugal
[2]LAQV/REQUIMTE. Faculdade de Farmácia. Universidade do Porto, Ciências Químicas, Porto, Portugal
[3]CISA. Centro de Investigação em Saúde e Ambiente- Escola Superior de Saúde- Politécnico do Port o, Saúde Ambiental, Porto, Portugal
[4]Laboratório Nacional de Referência de Infeções Gastrintestinais. Instituto Nacional de Saúde Dr. Ric ardo Jorge, Doenças Infeciosas, Lisboa, Portugal
[5]Faculdade de Ciências da Nutrição e Alimentação. Universidade do Porto, FCNAUP, Porto, Portugal

Backgrounds

Arsenic environmental contamination by anthropogenic activities, including in animal-farming management (e.g. coccidiostatics/pesticides/waste), may represent a long-term selective pressure driving the selection of multidrug-resistant (MDR) emergent *Salmonella* serotypes/clones. Diverse arsenic tolerance (AsT) mechanisms were described, although dispersion and association with a tolerance phenotype remains unknown in *Salmonella*.

Objectives

To study the occurrence of genes coding for arsenical efflux pumps and their implications in tolerance phenotypes in diverse *Salmonella* serotypes/clones.

Methods

284 *Salmonella* isolates (2000-2016; humans/foods/animal/environment) from 58 serotypes (including the most frequent: Enteritidis/n=16; Typhimurium/n=48; 4,[5],12:i:-/n=65; Rissen/n=15) were selected based in PFGE-type and different profiles of antibiotic-resistance or metal-tolerance (Cu/Ag/Hg/Te) (PMID-27118781). Screening of *arsB* and *acr3* was performed by PCR/sequencing. $MIC_{Na2HAsO4}$ were determined in aerobic and anaerobic atmospheres by agar dilution method.

Conclusions

A high frequency of AsT genes was found (150/284-53%) involving diverse serotypes (30/58-52%): *arsB* (25%-n=70; 8 serotypes) or *acr3* (29%-n=82; 24 serotypes). The *arsB* was almost restricted to the emergent pig-associated MDR “European clone” of *S*.4,[5],12:i:- (n=36/36-100%) and *S*.Typhimurium (n=25/25-100%). The *acr3* was highly dispersed, including in two emerging pig-associated serotypes *S*.Rissen (n=15/15-100%) and *S*.Derby (n=8/8-100%). Phenotypic assays showed higher $MIC_{Na2HAsO4}$ in isolates carrying *arsB* (MIC_{50}=>128mM) or *acr3* (MIC_{50}=8mM) than those without these genes (MIC_{50}=2mM) in both aerobic/anaerobic conditions. AsT isolates also carried frequently (n=105/150-70%; “European clone” and Rissen plus 9 serotypes) copper, silver ± mercury, tellurium tolerance genes. Occurrence of AsT, particularly among emergent MDR and copper/silver tolerant pig-associated *Salmonella* clones circulating in Europe, might contribute for their adaptation to food-animal farm environments contaminated with diverse metals and oxygen levels.

FEMS7-2612
Environmental Microbiology/Microbial Ecology /Microbial Communities

MARINE BIOMONITORING: PREDICTING BIOTIC INDICES FROM EDNA METABARCODING DATA USING SUPERVISED MACHINE LEARNING

T. Cordier[1], P. Esling[2], F. Lejzerowicz[1], J. Pawlowski[1]
[1]University of Geneva, Department of genetics and evolution, Geneva, Switzerland
[2]University Pierre and Marie Curie, IRCAM, Paris, France

Backgrounds

Monitoring diversity of benthic organisms is an important tool for environmental impact assessment of anthropogenic activities in marine environment. Traditional benthic monitoring involves the morpho-taxonomic identification of thousands of manually sorted macrofaunal specimens, which is extremely time-consuming and taxonomic expertise demanding. High-throughput sequencing of environmental DNA (eDNA metabarcoding) offers an alternative to describe biological communities. However, an important fraction of eDNA sequences are new to science or belong to species of unknown ecology, which strongly limits their use for inferring biotic indices.

Objectives

To overcome these limitations, we investigated the possibility of using supervised machine learning (SML) algorithms to build predictive models from eDNA metabarcoding data targeting groups that are not commonly used for benthic monitoring.

Methods

We tested our approach on benthic foraminifera, a group of unicellular eukaryotes known to be sensitive to organic enrichment associated with marine aquaculture.

Conclusions

Our study conducted on five salmon farms in Norway show that SML tools applied to foraminiferal metabarcoding data allow the building of accurate predictive models, leading to similar biotic indices values than the ones derived from macrofaunal inventories. Although the routine application of SML still requires some adjustments and validations, we argue that such approaches could be used to overcome and even bypass the cost and time demanding macrofaunal approach in future biomonitoring.

FEMS7-2749
Environmental Microbiology/Microbial Ecology /Microbial Communities

WASTEWATER EFFLUENTS BACTERIAL COMMUNITY INFLUENCE THE MICROBIOME AND THE RESISTOME OF THE RECEIVING WATER

G. Corno[1], Y. Yang[2], A. Di Cesare[1], D. Fontaneto[1], E. Eckert[1]
[1]CNR-National Research Council of Italy, Institute of Ecosystem Study - Microbial Ecology Group, Verbania, Italy
[2]Sun Yat-sen University, School of Marine Sciences, Guangzhou, China

Backgrounds

Wastewater treatment plants (WWTPs) are a hot-spot for the spread of antibiotic resistance genes (ARGs) into the environment. Several studies targeted the selection and persistence of ARGs in WWTPs applying different disinfection technologies. Still, little is known on the fate of resistances once they are spread into the environment with the treated effluents.

Objectives

This is the first study on the fate of bacteria and ARGs released from different WWTPs once in contact with the resident microbial communities of the receiving water bodies. The result of complex ecological interactions concomitantly acting on the newly formed mixed communities gives an overview and allows speculations on the potential risk of ARGs spread under different environmental conditions.

Methods

Experimental mixed communities were designed mimicking the impact of different (in size and disinfection) WWTP effluents on the communities of various receivers (lake, river). Bacterial abundance and phenotype was assessed by flow-cytometry and microscopy. Bacterial communities composition and their relative resistome (1208 different ARGs) were assessed by full-genome metasequencing.

Conclusions

The fate of the bacteria from the WWTPs depended by the disinfection applied but even more on the ecological stability of the microbial community of the receiving system. ARGs released into the environment could established in the newly formed communities and those already present into the resident community surprisingly increased especially when the introduction of WWTP effluents was limited (e.g. 10% of the final water volume). This study demonstrated how the impact of the WWTP effluents is heavily influenced by the ecological conditions of the resident microbial community.

FEMS7-0168
Environmental Microbiology/Microbial Ecology /Microbial Communities

ISOLATION AND CHARACTERIZATION OF FUNGAL STRAINS WITH CELLULOLYTIC ACTIVITY ON A 19TH CENTURY COLLECTION OF ART

C. Coronado Ruiz[1,2], *R. Avendaño*[1], *G. Conejo*[3], *E. Escudero*[2,4], *P. Chaverri*[2,4], *M. Chavarría*[1,2,3]
[1]*CeNAT-CONARE, Centro Nacional de Innovaciones Biotecnológicas CENIBiot, 1174-1200 San José, Costa Rica*
[2]*Universidad de Costa Rica, Centro de Investigaciones en Productos Naturales CIPRONA, 11501-2060 San José, Costa Rica*
[3]*Universidad de Costa Rica, Escuela de Química, 11501-2060 San José, Costa Rica*
[4]*Universidad de Costa Rica, Escuela de Biología, 11501-2060 San José, Costa Rica*

Backgrounds

The archive of the University of Costa Rica holds a 19th century French collection of drawings and lithographs in which the biodeterioration by fungi is notorious. Due to nutritional limitations in which these fungi grew up, it is believed that the isolated strains possess the ability to degrade cellulose.

Objectives

Our goal was to isolate and identify the fungal strains responsible for the biodegradation of the 19th century collection and determine their cellulolytic activity.

Methods

Fungi were isolated using potato dextrose agar (PDA) and carboxymethyl cellulose (CMC). Identification of fungi was assessed by DNA sequencing complemented with morphological analysis. Finally, assays for cellulolytic activity was conducted by using Congo red dye.

Conclusions

Twenty different species were isolated. By sequence analysis (ACT and ITS regions), 90% of all strains were identified correctly to the species level belonging mainly to the genera *Aspergillus, Trichoderma, Cladosporium, Penicillium, Calostilbe, Chaetomium* and *Arthrinium*. There was no register in the Gene Bank for the sequence of two samples; consequently, they were identified through morphological analysis as new species of the genera *Periconia* and *Coniochaeta*. Qualitative tests show that the fungus collection present important cellulolytic activity.

FEMS7-3088
Environmental Microbiology/Microbial Ecology /Microbial Communities

REGULATORS AND FACTORS INFLUENCING THE MOBILITY OF PLANT PATHOGENIC BACTERIA OF THE GENUS DICKEYA

N. Cotte-Pattat[1], *V. Utzinger*[2]
[1]*Université Claude Bernard Lyon 1, Biology, VILLEURBANNE, France*
[2]*INSA de Lyon, Biosciences, Villeurbanne, France*

Backgrounds

Bacteria of the genus *Dickeya* are causal agents of soft-rot diseases on various plants, including economically important crops. Most phytopathogenic bacteria are mobile and this ability contributes to plant colonization and virulence. *Dickeya* can move either by 'swimming' in a fluid environment, or by 'swarming' on a surface. While swimming is an individual cell behavior, swarming depends on remarkable collective behavior.

Objectives

Our objective was to identify the factors and regulators influencing the swarming and swimming motility of the *D. dadantii* strain 3937.

Methods

Using conventional media with low agar concentrations, *D. dadantii* 3937 showed only moderate mobility. Analysis of different growth conditions showed a major influence of the carbon source on swarming and swimming. It was previously observed that the *D. dadantii* motility greatly increased after inactivation of PecS, a repressor controlling various virulence factors in response to unknown condition(s). Thus, we analyzed the swimming and swarming motility of a collection of mutants containing an inactivated transcriptional regulator.

Conclusions

Easily metabolizable carbon sources are the most efficient sugars to induce swarming; they could bring enough energy for this process. Swimming was differently influenced depending on carbon sources, probably due to chemotactic responses to some of them (galactose, ribose, xylose…). In addition to PecS, several regulators were found to affect (up or down) the swimming and/or swarming motility. This ability is controlled by a complex set of regulators, in response to environmental conditions that could be encountered by *Dickeya* during the plant infection.

FEMS7-1603
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROBIOTA CHARACTERIZATION AND VOLATILE AND NON-VOLATILE LIPOPHILIC FRACTION ANALYSIS OF BITTO STORICO CHEESE PRODUCED IN SIX DIFFERENT ALPINE PASTURES.

P. Cremonesi[1], F. Turri[1], G. Gandini[2], G. Battelli[3], M. Severgnini[4], F. Pizzi[1]
[1]Consiglio Nazionale delle Ricerche, Institute of Agricultural Biology and Biotechnology, Milan, Italy
[2]Università degli Studi di Milano, Department of Veterinary Medicine, Milan, Italy
[3]Consiglio Nazionale delle Ricerche, Institute of Sciences of Food Production, Milan, Italy
[4]Consiglio Nazionale delle Ricerche, Institute of Biomedical Technologies, Milan, Italy

Backgrounds

The Bitto Storico is an alpine heritage cheese made with raw milk and produced during the summer season in the Orobic Alps (Central Alps, Northern Italy). The cheese-making process depends on local traditions: freshly milked cow's milk is added to fresh goat's milk (10-20%), then production starts immediately in the "calècc" (itinerant dairy). The use of feeds and silage for animal feeding and the use of probiotics during curdling are prohibited thus the organoleptic variability of the cheese depends on local bacteria that change according to pastures and environment. These conditions guarantee a variability of organoleptic characteristics, probably associated to different microbial communities, highly appreciated by consumers.

Objectives

The objectives were (i) the characterization of the microbial variability of the Bitto Storico cheese by Next Generation Sequencing, and (ii) the analysis of the volatile and non-volatile lipophilic fractions.

Methods

Samples were collected from 54 Bitto Storico cheeses produced in six different Alpine pastures, in three geographical areas (Val Gerola, Valli del Bitto, Valle Brembana), during 2015 summer season. Bacterial DNA was extracted using an optimized protocol and 16S rRNA gene amplicons on V3-V4 region analyzed by Miseq (Illumina). The lipophilic fraction was characterized by means of Solid Phase Extraction-Gas Chromatography-Mass Spectrometry (SPME-GC-MS), the fatty acid profile by the High Resolution Gas Chromatography (HRGC).

Conclusions

Preliminary results showed a microbial community mainly dominated by *Firmicutes* with a high abundance of *Streptococcaceae* and *Lactobacillaceae* and low presence of *Entrococcaceae*. The volatile metabolome displayed a variability consistent with the dairy products of raw milk.

FEMS7-1032
Environmental Microbiology/Microbial Ecology /Microbial Communities

GLYCEROL METABOLISM INDUCES BIOFILM FORMATION AT THE AIR-LIQUID INTERPHASE IN LISTERIA MONOCYTOGENES

N. Crespo Tapia[1], M.W. den Besten[1], T. Abee[1]
[1]Wageningen University & Research, Food Microbiology, Wageningen, The Netherlands

Backgrounds

Listeria monocytogenes is a microaerophilic food-borne pathogen that can grow as a biofilm on the surface of food-processing equipment. Bacterial biofilms vary greatly in structure and characteristics, and can be influenced by a wide range of environmental conditions.

Objectives

Although motile aerobic bacteria have been described to form biofilms at the air-liquid interphase of cell cultures, to our knowledge, this type of biofilm has never been described in *L. monocytogenes*. In this study we report *L. monocytogenes* biofilm formation at the air-liquid interphase of aerobically grown cultures, and that this phenotype is specifically induced when the media is supplemented with glycerol as a carbon/energy source.

Methods

A link with aerobic glycerol metabolism was confirmed by the analysis of *L. monocytogenes*' performance under anaerobic conditions, where biofilm production was reduced to the same level as the non-supplemented control, and it was located at the bottom of the well. Planktonic growth performance, metabolic activity assays and HPLC measurements of glycerol consumption over time showed that glycerol utilization in *L. monocytogenes* is restricted to growth under aerobic conditions. Additional motility assays revealed the induction of aerotaxis in the presence of glycerol. Gene expression analysis will provide further insight in parameters involved in glycerol metabolic pathway(s), aerotaxis and biofilm formation.

Conclusions

We hypothesize that the formation of biofilms at the air-liquid interphase is dependent on glycerol-induced aerotaxis towards the surface of the culture, where *L. monocytogenes* has access to higher concentrations of oxygen, and is therefore able to utilize this compound as a carbon source.

FEMS7-1513
Environmental Microbiology/Microbial Ecology /Microbial Communities

DETECTION AND METABOLIC MONITORING OF MICROBIAL CONSORTIA IN BENTONITE CLAY

S. Crook[1], M. Hausner[1], M. Kroukamp[1]
[1]Ryerson University, Department of Chemistry and Biology, Toronto, Canada

Backgrounds

Extreme environments present unique challenges to microbiologists. One such example of an extreme environment is bentonite clay. This natural material, slated to serve as the primary environmental barrier in the Deep Geologic Repository for spent nuclear waste, has been well characterized both physically and chemically. However, the ultra-oligotrophic and hygroscopic nature of bentonite, along with low biomass have presented hurdles in characterizing the microbial communities present, especially in the realm of molecular tools. The metabolic products of sulfate reducers and other microbes could potentially have profound effects on the proposed DGR design by influencing rates of corrosion on surfaces integral to the containment of nuclear waste. Past studies have attempted to identify relevant bacterial and eukaryotic organisms which may affect the proposed DGR design. However, the presence of archaeal populations and the role they may play has yet to be investigated.

Objectives

This study seeks to identify microbial populations native to bentonite clay and characterize the metabolic properties of these organisms.

Methods

The presence of microorganisms was investigated through the use of both cultivation and DNA-based techniques. To characterize the metabolic properties of sulfate reducing archaea/bacteria and methanogens present, anaerobic serum bottles containing bentonite and selective media were inoculated with enrichments from a bentonite sample. Sulfate, sulfite, acetate and methane concentrations were monitored.

Conclusions

Given sufficiently favorable conditions, microbially driven processes appear to occur which could potentially affect the proposed DGR design. This research furthers the understanding of the role played by microorganisms in bentonite and other extreme environments.

FEMS7-2083
Environmental Microbiology/Microbial Ecology /Microbial Communities

BACTERIAL DIVERSITY SHIFT DETERMINED BY DIFFERENT DIETS IN THE GUT OF THE SPOTTED WING FLY DROSOPHILA SUZUKII IS PRIMARILY REFLECTED ON ACETIC ACID BACTERIA

E. Crotti[1], V. Vacchini[1], E. Gonella[2], E.M. Prosdocimi[1], F. Mazzetto[2], B. Chouaia[1], M. Callegari[1], F. Mapelli[1], M. Mandrioli[3], A. Alma[2], D. Daffonchio[4]
[1]Università degli Studi di Milano, Dipartimento di Scienze per gli Alimenti- la Nutrizione e l'Ambiente, Milano, Italy
[2]Università degli Studi di Torino, Dipartimento di Scienze Agrarie- Forestali e Alimentari, Grugliasco, Italy
[3]Università di Modena e Reggio Emilia, Dipartimento di Scienze della Vita, Modena, Italy
[4]King Abdullah University of Science and Technology,
Biological and Environmental Sciences and Engineering Division, Thuwal, Saudi Arabia

Backgrounds

The role of diet in shaping the gut microbiota was evaluated in different animal models, including insects. *Drosophila* flies host an inconstant microbiota among which acetic acid bacteria (AAB) are important components capable of modulating immune response and the insect development.

Objectives

We characterized the bacterial and AAB communities associated to the spotted wing fly *Drosophila suzukii*, an invasive pest, by studying the same insect population separately grown on fruit-based or non-fruit artificial diet.

Methods

Prevalence of AAB in the insect gut was investigated by specific PCR. AAB capability to colonize the gut was estimated by fluorescent *in situ* hybridization and recolonization experiments with green fluorescent protein (Gfp)-labelled strains. Bacterial communities of individuals reared on the two diets were evaluated by pyrosequencing, analyzing the V1-V3 region of the 16S rRNA gene.

Conclusions

AAB massively colonized the insect gut with infection rates of 90 and 92% in fruit-fed and artificial diet-fed individuals, respectively. 16S rRNA gene pyrosequencing showed a differentiation of the bacterial microbiota of guts from insects fed with the two diets. The exclusion of AAB-related OTUs from the analysis showed a lack of the clustering pattern, suggesting that the diet-based diversification of the community is primarily reflected on AAB components. AAB alpha-diversity was also influenced by diet. Data suggested an AAB role in the gut response of *D. suzukii* to diet modification.

FEMS7-0088
Environmental Microbiology/Microbial Ecology /Microbial Communities

BIOCONTROL OF CHESTNUT BLIGHT: (IN)STABLE INFECTION OF THE CHESTNUT BLIGHT FUNGUS BY CRYPHONECTRIA HYPOVIRUS 1

M. Curkovic-Perica[1], M. Jezic[1], V. Karin[1], M. Idzojtic[2], K. Sotirovski[3], M. Risteski[3], D. Rigling[4], S. Prospero[4], M. Kolp[5], M. Double[6]
[1]Faculty of Science- University of Zagreb, Department of Biology, Zagreb, Croatia
[2]Faculty of Forestry- University of Zagreb, Department of Forest Genetics- Dendrology and Botany, Zagreb, Croatia
[3]Saints Cyril and Methodius University of Skopje, Faculty of Forestry, Skopje, FYR Macedonia
[4]WSL Swiss Federal Institute for Forest- Snow and Landscape Research, Department of Phytopathology, Birmensdorf, Switzerland
[5]Michigan State University, Plant Biology, East Lansing, USA
[6]West Virginia University, Division of Plant and Soil Sciences, Morgantown, USA

Backgrounds

Chestnut blight is a disease caused by the plant pathogenic fungus *Cryphonectria parasitica*, which induces bark lesions (cankers) on stems and branches of chestnut (*Castanea*) species. The pathogen is successfully controlled by a double-stranded RNA mycovirus *Cryphonectria hypovirus* 1 (CHV1), which reduces virulence and reproductive capacities of the fungus, a phenomenon called hypovirulence. If the virus is introduced naturally or artificially into an active canker caused by virulent *C. parasitica* strain(s), canker expansion ceases and the canker calluses resulting in a so-called healed canker. High prevalence of CHV1 in *C. parasitica* populations often results in the recovery of chestnut forests. Therefore, it is important that CHV1-infected fungal strains persist on chestnut trees over years.

Objectives

The aim of this study was to investigate the persistence of hypovirulent *C. parasitica* strains in completely healed, callused chestnut blight cankers.

Methods

Bark samples were collected in seven blighted chestnut stands (six in Europe and one in North America) from more than 100 healed bark cankers.

Conclusions

The frequency of hypovirulent *C. parasitica* strains in sampled cankers varied substantially between different populations. Virulent *C. parasitica* strains were more frequent than hypovirulent ones in healed cankers, which was unexpected because CHV1-infected strains are thought to promote canker healing. The relationship between virulent and hypovirulent fungal strains in healed cankers appears to be highly dynamic. We suspect that in some cases the loss of vigor by hypovirulent *C. parasitica* strains leaves a niche for virulent ones to re-invade the already healed cankers.

FEMS7-2524
Environmental Microbiology/Microbial Ecology /Microbial Communities

DIVERSITY OF EXTRACHROMOSOMAL REPLICONS IN BACTERIA OF THE GENUS PARACOCCUS (ALPHAPROTEOBACTERIA)

J. Czarnecki[1], P. Decewicz[1], M. Szuplewska[1], A. Pawłot[1], R. Lasek[1], E. Prochwicz[1], M. Puzyna[1], A. Brozio[1], K. Kotecka[1], P. Dymek[1], Ł. Dziewit[1], D. Bartosik[1]
[1]Institute of Microbiology, Department of Bacterial Genetics, Warsaw, Poland

Backgrounds

Genomes of many *Alphaproteobacteria* (including members of the genus *Paracoccus*) comprise numerous extrachromosomal replicons (ERs) of diverse properties and different evolutionary history. The ERs are divided into non-essential plasmids and essential chromids, which contain house-keeping genes of chromosomal origin and are necessary for the viability of their hosts.

Objectives

We aimed to identify and analyze replication systems (REPs) of all ERs occurring in 18 strains of *Paracoccus* spp. We examined phylogenetic relationships of the REPs and analyzed genetic content of individual replicons in terms of the presence of: (i) the core genes conserved in all *Paracoccus* spp. genomes, and (ii) adaptive genes important in the natural niches of the tested strains. We assumed to obtain valuable data on the content, properties, distribution and directions of horizontal transmission of ERs in the genus *Paracoccus*.

Methods

In silico sequence analyses (construction of phylogenetic trees, determination of the core genome, search for adaptive genes) were performed using standard on-line available bioinformatic tools. Standard genetic techniques were used to clone REPs of individual ERs in a form of shuttle plasmids.

Conclusions

We characterized over 50 ERs in *Paracoccus* spp. Majority of them contained *dnaA-like*, *repB* and *repABC* REPs. Most of the strains carried two large ERs, with *dnaA-like* and *repB* REPs, essential for normal growth of their host strains. Other replicons (including those with *repABC* replication modules) were neither conserved nor essential, which suggests their relatively recent lateral acquisition.

FEMS7-1814
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROBIAL GROWTH FACTORS AFFECTING THE EXOPOLYMER PRODUCTION BY RHODOCOCCUS OPACUS

M. Czemierska[1], *A. Szcześ*[2], *A. Jarosz-Wilkołazka*[1]
[1]*Maria Curie Skłodowska University, Department of Biochemistry, Lublin, Poland*
[2]*Maria Curie Skłodowska University, Department of Physical Chemistry - Interfacial Phenomena, Lublin, Poland*

Backgrounds

Microorganisms such as fungi, bacteria and yeasts are able to synthesise exopolymers with many biologically important properties. Particularly valuable is their ability to purify the water from dyes, heavy metals, and colloidal particles by flocculation. This process can be applied for the removal of the suspended particles, causing their aggregation and sedimentation. Addition of natural polymers to the solution significantly accelerates this phenomenon and makes it safe for human health and environment. Therefore, there are many studies about obtaining new products to find the most effective and relatively inexpensive source of biopolymers with flocculating activity.

Objectives

The flocculation efficiency of exopolymers depends on many different factors and is strictly correlated with culture growth conditions. Therefore, it is crucial to optimise bacterial growth factors to receive exopolymers with high flocculating activity and possibly by the most efficient methods of extraction.

Methods

The optimum culture conditions were determined using different variants of culture, such as carbon and nitrogen sources, salts addition and pH value of the medium. Supernatants obtained from *Rhodococcus opacus* culture broths were tested for the flocculating activity in the presence of kaolin suspension.

Conclusions

The synthesis of the exopolymers with flocculating activity by microorganisms depends on multiple factors such as carbon and nitrogen sources, the pH value of medium and salts addition to culture growth. Determination of microbial growth conditions allows to obtain the products with high flocculating activity.

This work was supported by the National Science Centre based on decision number 2016/21/N/NZ9/02309.

FEMS7-2726
Environmental Microbiology/Microbial Ecology /Microbial Communities

EVALUATION OF PLANT GROWTH-PROMOTING EFFECTS AND ANTIBACTERIAL PROPERTIES OF ENDOPHYTIC BACTERIA ASSOCIATED WITH TOMATO PLANTS

S. Dahikar[1]
[1]Sanjivani Arts- Commerce and Science College- Kopargaon, Department of Microbiology, Kopargaon, India

Backgrounds

Endophytic bacteria are widely studied because of their plant-growth-promoting benefits. They use various direct and indirect mechanisms to promote plant growth.

Objectives

The objective of these experiments were to select and characterized endophytic bacteria isolated from tomato stems, and to investigate the plant growth-promoting effect to develop systems to improve plant health and crop productivity based on microbial inoculation and as a biocontrol agents of tomato bacterial pathogens.

Methods

This study was conducted to select and characterize endophytic bacteria isolated from healthy tomato stems and to test their ability to promote plant growth and suppress bacterial diseases. The isolates were analyzed for a number of different PGP traits: organic acids (OA), indole acetic acid (IAA), ACC deaminase, and siderophore production. The antibacterial properties of the isolated strains were determined against *Ralstonia solanacearum*, *Xanthomonas campestris, Pseudomonas syringae* and *Clavibacter michiganensis.*

Conclusions

In the present study, a total of 17 strains of endophytic bacteria were isolated from tomato roots namely *Microbacterium* sp., *Bacillus* sp., *Rhizobium* sp., *Rhodococcus* sp., *Agrobacterium* sp., *Rhizobium* sp. A high percentage of the isolated strains tested positive for the following PGP traits: 67% were able to produce OA, 83% IAA, 76% ACC deaminase, and 81% siderophores. Endophytic bacteria which show significant antibacterial activity against *R. solanacearum*. The present studies strongly suggest that the endophytic bacteria characterized in this study could be successfully used to promote plant growth and act as biocontrol agents in tomato plants.

FEMS7-0619
Environmental Microbiology/Microbial Ecology /Microbial Communities

MULTIDRUG RESISTANT CTX-M TYPE PRODUCING ESCHERICHIA COLI OF PHYLOGENETIC GROUP B2 IN INTESTINAL COLONIZATION OF HEALTHY PORTUGUESE CATTLE

J. Dantas Palmeira[1,2], *H. Ferreira*[1,2]
[1]*University of Porto, Laboratory of Bacteriology, Porto, Portugal*
[2]*UCIBIO, REQUIMTE, Porto, Portugal*

Backgrounds

Background: Intestinal colonization of food-producing animals with extended-spectrum beta-lactamase (ESBL) producers represents a public health concern. *Escherichia coli,* an important commensal of the intestinal tract of animals is responsible for the spread of antimicrobial resistance.

Objectives

Objective: This study aimed to the characterization of CTX-M-type ESBL and phylogenetic grouping of multidrug-resistant (MDR) ESBL-producing *Escherichia coli* in feces of healthy bovine from Portugal.

Methods

Material/methods: Selection of the isolates obtained from fecal samples of bovine in Portugal, was performed on MacConkey agar with antibiotics, with previous incubation in TSB. Susceptibility testing was achieved by disk-diffusion-method according to the CLSI. Presumptive identification was performed by CHROMagar-Orientation. ESBL-producers were screened by the double-disk-synergy-test. ESBL coding genes were screened by PCR. CTX-M-type producing *E.coli* isolates were selected for further study. Phylogenetic groups were determined by PCR.

Conclusions

Results/Conclusions: From 117 cattle fecal samples, 470 *Enterobacteriaceae* isolates were obtained, showing a total of 392 MDR isolates, 227 (48.30%) *Escherichia coli* isolates were ESBL-producers (EC-ESBL), from these, 223 were CTX-M-producers. The EC-ESBL-CTX-M gene profile was: *bla*$_{\text{CTX-M}}$Group1 (87%); *bla*$_{\text{CTX-M}}$Group9 (12.1%); *bla*$_{\text{CTX-M}}$Group1+*bla*$_{\text{CTX-M}}$Group9 (0.9%). Phylogenetic group A was prevalent with 150 (67.3%) of EC-ESBL-CTX-M, 61 (27.4%) B1, 9 (4%) B2 and 3 (1.3%) D.
Results show prevalence of *bla*$_{\text{CTX-M}}$ EC-ESBL (98.24%) presenting a MDR phenotype and phylogenetic group A colonizing these animals. Relevant phylogenetic group B2 isolates presenting *bla*$_{\text{CTX-M}}$ were detected. The presence of *bla*$_{\text{CTX-M}}$ EC-ESBL in animal intestinal colonization shows a reservoir of multidrug-resistant microorganisms relevant in environmental and community spread of CTX-M-type ESBL.

FEMS7-2156
Environmental Microbiology/Microbial Ecology /Microbial Communities

MULTI-DRUG RESISTANT (MDR) CTX-M-15-PRODUCING ESCHERICHIA COLI FROM INTESTINAL COLONIZATION OF HEALTHY PETS IN THE NORTH OF PORTUGAL

C. Araújo[1], J. Dantas Palmeira[1,2], H. Ferreira[1,2]
[1]University of Porto, Biological Sciences, Porto, Portugal
[2]REQUIMTE, UCIBIO, Porto, Portugal

Backgrounds

Spread of *Escherichia coli* MDR-ESBL (EC-ESBL) producers has become a serious global problem in terms of public health. Intestinal colonization of pets with ESBL producers is problematic and contributes to antimicrobial resistance dissemination.

Objectives

The aim of this study was detection of MDR ESBL-producing *Enterobacteriaceae* in intestinal colonization of healthy pets in Portugal.

Methods

Nine companion animal fecal samples were enriched in TSB. Selection was obtained in MacConkey agar with antibiotics. Antimicrobial susceptibility was determined by disk-diffusion-method by EUCAST. ESBL producers were detected by double-disk-synergy-test. Presumptive identification was done by CHROMagar orientation. EC-ESBL were selected for detection of *bla*$_{\text{CTX-M}}$ groups (group1 positives were screened for *bla*$_{\text{CTX-M-15}}$), phylogenetic groups and non-beta-lactam resistance genes were studied by PCR.

Conclusions

A total of 50 *Enterobacteriaceae* were selected showing high resistance profile: 82% resistant to amoxicillin, 42% to amoxicillin+clavulanic acid, 14% to cefotaxime, 58% to tetracycline, 56% to ciprofloxacin and 42% to sulphamethoxazole+trimethoprim. Thirty-nine (78%) isolates showed MDR profile. A total of 6 isolates were MDR-ESBL-producing *E. coli* (EC-ESBL), of these, 4 carried the *bla*$_{\text{CTX-M-15}}$ gene. Phylogenetic classification showed 2 EC-ESBL group A, 1 B1 and 3 D. EC-ESBL showed diversity of non-beta-lactam resistance genes: 2 ***aac6'Ib-cr***, 2 *qnrB*, 6 *parC*, 5 *gyrA* and 5 *tetA* genes.

The results substantiate the occurrence and ongoing spread of various ESBL-producing, multi-resistant *E.coli* in healthy pets. These animals can act as ESBL-producers reservoir for humans highlighting the idea that antibiotic resistance is a public health problem that needs to be managed in a one-health perspective, encompassing human and animal health.

FEMS7-2229
Environmental Microbiology/Microbial Ecology /Microbial Communities

DIVERSITY OF HALOPHILIC BACTERIA IN NAMAKDAN HYPERSALIN CAVE IN QESHM

M. Darabi[1], N. zamani[1], M. mehrshad[1], M.A. amoozegar[1]
[1]Tehran university faculty of science, microbiology, Tehran, Iran

Backgrounds

Namakdan Cave in Qeshm island Geopark; south of Iran, is the longest salt cave in the world with 6850m length. In persian, the word "namak" means salt or Sodium Chloride which is the main component of Namakdan salt dome. Caves are dark and nutrient-limited environments. Microbial processes occurring in the absence of light have generally been considered insufficient to support ecosystem-level processes. bacterial diversity in caves is still rarely investigated.

Objectives

In the present study bacterial diversity in Namakdan salt cave has been investigated by culture dependent methods.

Methods

Sampling was carried out in October and Physico-chemical variables (e.g. temperature, conductivity and pH) were measured in situ. The salinity and pH of the samples were 32.07% and 7.1. In order to isolate halophilic bacteria Marine agar medium with 10% salinity was used. Total of 70 isolate were separated. 16S rRNA gene amplification, sequencing and analysis was performed for selected isolates and it could recognize members of Firmicutus and Bacteroidets phyla in *Paenibacillus, Aquibacillus, Paraliobacillus, Bacillus, Aliifodinibius* genus that need at least 2% salinity in their culture media. The optimal temperature range for growth of this bacteria is 30 to 34°C. most of them are rod shape and the rest are coccoid.

Conclusions

The present study is intended to determine the genetic map of Namakdan salt cave which will help us to identify unknown halophilic bacteria community of this unique environment and also stable the position of this Geopark in UNESCO.

FEMS7-0657
Environmental Microbiology/Microbial Ecology /Microbial Communities

VISIBLE LIGHT ASSISTED PHOTOCATALYTIC DISINFECTION OF MDR ESCHERICHIA COLI USING DOPED ZINC OXIDE NANOCOMPOSITES

S. Das[1], *S. Tripathy*[1]
[1]*KIIT University, School Of Biotechnology, Bhubaneswar- Orissa, India*

Backgrounds

With supplies of fresh water diminishing and population increasing, water scarcity is set to become a major global problem. Emerging pollutants including multi-drug resistant(MDR) bacteria possess risk for public health. Conventional treatment methods have been proven ineffective as far as their economy and performance is concerned. This necessitate an immediate need for alternative disinfection technology. Photocatalysis using semiconductor nano-composites have gained attention in recent time. Photocatalysis involves metal oxides in presence of water, which when illuminated with light generates reactive oxygen species (ROS) which serves detrimental to pathogenic bacteria.

Objectives

To Check the effect of solar-photocatalytic disinfection (PCD) on MDR *Escherichia coli* (environment isolate) using doped ZnO, To understand molecular disinfection mechanism and bacterial reactivation post disinfection (PD) and checking efficiency of the process with real water systems.

Methods

Synthesis of metal doped ZnO using chemical co-precipitation route. PCD and process optimization. Effect of different ROS scavenger on the rate of disinfection. Lipid peroxidation assay, LIVE/DEAD staining, Potassium ion leakage, Electron microscopy and DNA damage analysis to confirm the disinfection process. Validation of PCD with real water samples.

Conclusions

Solar-PCD was perfectly achieved (with 500mg/L Doped ZnO) within 90 minutes. Membrane damage, intracellular ion leakage and DNA damage was found to be the key mechanism of disinfection with no reactivation of *MDR E. coli* till 7 days post disinfection. H_2O_2 was the key species involved in solar-PCD of MDR *E. coli*. The process is validated in real water samples collected from different sources and is expected to have potential in real world application.

FEMS7-0399
Environmental Microbiology/Microbial Ecology /Microbial Communities

GENOME SCALE RECONSTRUCTION OF AROMATIC HYDROCARBON DEGRADATION PATHWAYS IN MARINOMONAS FUNGIAE STRAIN AN44T AND THEIR COMPARATIVE ANALYSIS

S. Das[1], *S. Zachariah*[1], *J. Badhai*[1]
[1]*Institute of Life Sciences, Department of Biotechnology, Bhubaneswar, India*

Backgrounds

Oceans are the final sink for almost all known aromatic pollutants that are produced on land. Bacteria have evolved complex metabolic pathways via various genetic mechanisms for the degradation of diverse aromatic compounds, using them as carbon and energy source. Thus, the possibilities of identifying marine microbes with novel genetic and metabolic abilities to degrade aromatic compounds are immense. However, the limited availability of information on genomic organization and mode of regulation/regulatory networks involved in aromatic compound degradation and assimilation in marine bacteria has impeded their genetic manipulation for proper biotechnological applications.

Objectives

Elucidation of aromatic compounds degradation pathways in a marine bacterium *Marinomonas fungiae* strain AN44^T.

Methods

Methods include BLAST search against KEGG database and MetaCyc pathways web-server, and genome comparison using MUMer.

Conclusions

Genome based screening revealed the presence of two central pathways involved in aerobic degradation of 3, 4-dihydroxybenzoate and 3, 4-hydroxyphenylacetate, and four peripheral pathways involved in degradation of ferulate, *p*-coumarate, caffeate and quinate. The 3, 4-dihydroxybenzoate degradation pathway was encoded by a 9.85 kb single operonic cluster composed of 11 genes, whereas the 3, 4-hydroxyphenylacetate degradation pathway was encoded by a 31.3 kb operonic region composed of 30 genes. Comparative analysis shows certain degree of heterogeneity in the DNA sequence to their corresponding counterparts in other pathways, indicating different evolutionary origins. This study provides important genetic information on the plasticity and diversity of bacterial catabolic pathways for degradation of specific aromatic compounds.

FEMS7-2830
Environmental Microbiology/Microbial Ecology /Microbial Communities

COMPARTMENTALIZATION AND ACTIVITY OF CLASS 1 INTEGRONS AND INCP-1 PLASMIDS IN RIVER SEDIMENTS IMPACTED BY URBAN AND INDUSTRIAL ANTHROPOGENIC PRESSURE

M. De La Cruz Barron[1], X. Bellanger[1], L. Mathieu[1], H. Guilloteau[1], C. Merlin[1]
[1]Université de Lorraine, Laboratoire de Chimie Physique et Microbiologie pour l'Environnement, Nancy, France

Backgrounds

The accumulation of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in natural environments is being increasingly highlighted, especially for aquatic ecosystems. Since MGEs such as class 1 integrons and IncP-1 plasmids are genetic structures that capture or mobilize ARGs, they are currently used as a proxy for estimating the relative abundance of ARGs in various environments.

Objectives

The aim of this study was to determine the relative abundance and activity of class 1 integrons and IncP-1 plasmids in various compartments of the Orne River, namely raw water, suspended material and sediments, and to identify environmental reservoirs of ARGs.

Methods

Abundances of class 1 integrons and IncP1 plasmids were estimated by qPCR from community DNA extracts. Then, the activity of such MGEs was explored using highly sensitive bacterial biosensor assays reporting the transcriptional activity of genes involved in their mobility.

Conclusions

Both MGEs appeared statistically enriched in suspended materials and in the top layers of sediments (recent deposits) compared to raw water. In sediments, MGEs abundance decreases with depth, excepted for discrete layers exhibiting a 2-3 order of magnitude increase, clearly indicating the existence of MGEs reservoirs. For the vast majority of sediment samples, the abundance of MGEs evolved independently from the local content in organic or metal pollutants, even the local community structures. Considering the fact that the dissemination of MGEs largely involved replicative processes, we speculate that abnormally elevated levels of MGEs may also result from an active dissemination of these elements, which is currently being explored using biosensors assays.

FEMS7-1403
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROBIAL SUCESSION DYNAMICS IN THE FOREFIELD OF BREIÐAMERKURJOKULL GLACIER (ICELAND)

A. De los Ríos[1], D. García-Angulo[2], J. Curiel[2], J. Durán[3], R. Ortiz-Alvárez[4], A. Torralba-Burrial[5], S. Heiðmarsson[6], S. Pérez-Ortega[7]
[1]Museo Nacional de Ciencias Naturales-CSIC, Biogeochemistry and microbial ecology, Madrid, Spain
[2]Museo Nacional de Ciencias Naturales-CSIC, Biogeography and global change, Madrid, Spain
[3]Center for Functional Ecology- University of Coimbra, Live Sciences, Coimbra, Portugal
[4]Centre for Advanced Studies of Blanes CEAB-CSIC, Continental Ecology, Blanes, Spain
[5]Cluster of Energy- University of Oviedo, Environment and Climate Change, Oviedo, Spain
[6]Icelandic Institute of Natural History- IS-600, Botany, Akureyri, Iceland
[7]Real Jardín Botánico-CSIC, Mycology, Madrid, Spain

Backgrounds

One key consequence of glacier recession, as effect of climatic change, is the creation of new habitats for colonization. In glacier forefields, primary succession occurs simultaneously in soils and rocks recently discovered offering a type of natural experiment in which temporal colonization dynamics can be analyzed.

Objectives

A chronosequence established at Breiðamerkurjökull Glacier forefield, was used as a framework to analyze primary microbial succession processes in subarctic regions. This outlet glacier stretches to southeast from Vatnajökull Glacier and has been dramatically retreating during the 20th century.

Methods

Soil samples from different succession stages were collected. Microbial community structure was analyzed by high-throughput amplicon sequencing. Potential microbial activity (microbial respiration, N mineralization) as well as different soil attributes were also measured in these samples.

Conclusions

Microorganisms play a fundamental role in the initial colonization of exposed soils after glacier ice retreat. They are the only colonizers at soils close to the glacier front and are functionally relevant in later successional stages. High-throughput amplicon sequencing of fungal and bacterial communities revealed that the structure of microbial communities from soils close to the glacier front considerably differed to that found in later successional stages. After a first abrupt change in community composition at the beginning of the succession, changes occur smoothly but showed a clear trend along the chronosequence. We found a significant decrease in soil pH, and a significant increase in the size of both, the soil organic matter and the organic and mineral N pools, in parallel with the succession process. Rates of microbial respiration and N mineralization also significantly increased with time of exposure after glacier retreat. Hence, our results demonstrate that primary succession along this chronosequence is accompanied by both a replacement of microbial taxa and changes in soil functionality.

FEMS7-2684
Environmental Microbiology/Microbial Ecology /Microbial Communities

METAGENOMIC ANALYSIS OF THE MICROBIOME IN THREE DIFFERENT BIOREACTOR CONFIGURATIONS APPLIED TO COMMERCIAL LAUNDRY WASTEWATER TREATMENT

T.P. Delforno[1], G.V.J. Lacerda[1], I.N. Sierra-Garcia[1], D.Y. Okada[2], T.Z. Macedo[3], M.B.A. Varesche[3], V.M. Oliveira[1]
[1]CPQBA, DRM, Paulínia, Brazil
[2]Division of Technology in Environment Sanitation, School of Technology, Limeira-SP, Brazil
[3]Engineering School of São Carlos - University of São Paulo,
Department of Hydraulics and Sanitation, São Carlos-SP, Brazil

Backgrounds

High concentrations of linear alkylbenzene sulfonate (LAS), an anionic surfactant, have been detected in the laundry wastewater. Thus, molecular biology tools are used to understand the taxonomies and pathways involved in anionic surfactant removal from biological reactors such as (fluidized bed (FBR), expanded granular sludge bed (EGSB) and up-flow anaerobic sludge blanket (UASB), focusing on new strategies for biodegradation.

Objectives

Therefore, this study characterized and compared three distinct biological reactors used for laundry wastewater treatment using whole genome shotgun metagenomics.

Methods

Three samples were collected from three different configurations of biological reactors (FBR, EGSB and UASB) applied to laundry wastewater treatment. The reactors were fed only with laundry wastewater diluted with a public water supply to obtain an influent LAS concentration of approximately 15 mg L^{-1}. Metagenomes were sequenced on the Illumina Hiseq platform and were analyzed using MG-RAST, STAMP and PAST software.

Conclusions

The EGSB and UASB reactors were more closely related based on taxonomic and functional profiles, likely due to similar granular sludge and procedures adopted to ensure anaerobic conditions. The EGSB and UASB reactors showed a predominance of methanogens and genes related to methanogenesis, with a prevalence of the acetoclastic pathway, in addition to the peripheral and central O_2-independent pathways for aromatic compound degradation. By contrast, FBR showed a dominance of aerobic microbiota and pathways for O_2-dependent aromatic compound degradation.Therefore, although the reactors showed similar surfactant removal levels, the microbial composition, functional diversity and aromatic compound degradation pathways were significantly distinct.

FEMS7-2459
Environmental Microbiology/Microbial Ecology /Microbial Communities

COMPARISON OF THE DISSEMINATION OF 16S RRNA METHYLTRANSFERASE GENES IN E. COLI FROM HUMAN AND NATURAL EFFLUENTS USING WHOLE GENOME SEQUENCING

J.F. Delgado Blas[1], C.M. Ovejero[1], A. Hoefer[1], W. Calero-Caceres[2], M. Muniesa[2], M.P. Garcillan-Barcia[3], F. De La Cruz[3], B. Gonzalez-Zorn[1]
[1]Faculty of Veterinary Medicine and VISAVET Complutense University of Madrid, Animal Health Department, Madrid, Spain
[2]Faculty of Biology University of Barcelona, Microbiology Department, Barcelona, Spain
[3]University of Cantabria, Institute of Biomedicine and Biotechnology of Cantabria, Santander, Spain

Backgrounds

The environment plays a critical role in the dissemination of antibiotic resistance genes. The comprehension of the human impact on the acquisition and evolution of these genes is essential to mitigate the burden of antimicrobial resistance (AMR). Whole genome sequencing (WGS) has demonstrated to be highly useful to reveal the epidemiological elements governing the spread of AMR.

Objectives

The aim of this work was to determine the human influence on the ecology of 16S rRNA methyltransferase genes, which confer high resistance to amynoglicosides, by comparing *E. coli* from natural and human effluents using WGS.

Methods

A total of 43 *E. coli* isolates resistant to amynoglicosides (25 from two waste water treatment plants and 18 from two Spanish rivers near Barcelona) were fully sequenced by Illumina technology (MiSeq). The consequent analysis was performed by the Center for Genomic Epidemiology services. The structure of the methyltransferase gene-carrying plasmids was obtained using PLACNET.

Conclusions

We have identified two *E. coli* sequence types (ST479 and ST632) highly disseminated in waste water, which harbored the methyltransferase gene *rmtB*. However, the isolates recovered from river samples belonged to diverse STs, and all of them carried the methyltransferase gene *armA*. Furthermore, sewage isolates only shared an IncFII plasmid, while river isolates had a common IncHI2 plasmid. PLACNET analysis critically contributed to understand the resistance gene dynamics in both natural and human environments. These results show the impact of the human activity, which forces the selection of specific genes and plasmids associated to certain STs.

FEMS7-2326
Environmental Microbiology/Microbial Ecology /Microbial Communities

THERMOPHILES REMAIN ACTIVE IN DIFFERENT SOIL ENVIRONMENTS

J.A. Delgado[1], *E. Gómez*[1], *J.M. González*[1]
[1]*Institute of Natural Resources and Agrobiology of Seville IRNAS, Biogeochemistry- Plant and Microbial Ecology, Seville, Spain*

Backgrounds

Microbial activity at high temperatures on the soil surface has not received the importance it deserves. Recent research has shown the presence of thermophilic bacteria in soils, and the *Geobacillus* genus is the most characteristic representant. It has been proposed that in temperate soils these thermophiles can be found as vegetative cells and they can be potential participants of soil biogeochemical reactions. It must be considered that hight temperature events are frequently observed at medium and low latitudes, providing with temporal niches for thermophiles to grow in upper soil layers. Nevertheless, thermophiles must survive low temperature periods which are dominant at high latitudes.

Objectives

This study suggests that soil thermophiles remain active during long periods even at relatively low temperatures common in most soils.

Methods

To answer this question we will quantify the activity of these thermophilic cells in comparison with mesophiles in the same environments using molecular and physiological methods. Our results directly contribute to evaluate the behaviour of soil thermophiles in a variety of environments and conditions.

Conclusions

The relevance of soil thermophiles will be highlighted because they are able to contribute with highly significant activity profiles and low, but steady, maintenance strategies.

FEMS7-0435
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROBIAL BIODEGRADATION OF CHLOROETHENES IN GROUNDWATER USING NEXT GENERATION MOLECULAR AND STABLE ISOTOPE ANALYSES

J. DENONFOUX[1], J. HELLAL[2], G. IMFELD[3], L. HERMON[2,4], S. FERREIRA[1], S. VUILLEUMIER[4], C. JOULIAN[2]
[1]GENOSCREEN, Service Recherche- Développement et Innovation- Communautés microbiennes, LILLE, France
[2]BRGM, Département Eau Environnement Ecotechnologies, Orléans, France
[3]UMR 7517- Université de Strasbourg/EOST- CNRS,
Laboratoire d'Hydrologie et de Géochimie de Strasbourg LHyGeS, Strasbourg, France
[4]UMR 7156 UNISTRA - CNRS- Université de Strasbourg,
Department of Microbiology- Genomics and the Environment, Strasbourg, France

Backgrounds

Chlorinated hydrocarbons are widespread contaminants in groundwater and subsurface ecosystems due to their massive use as industrial solvents in the last decades. Their hydrophobic properties have led to their accumulation and persistence in aquifers, while their high toxicity and carcinogenicity pose serious risks for human health and the environment. Microbial biodegradation of halogenated hydrocarbons represents a promising dissipation process for rehabilitating polluted sites, although it requires diagnostic tools relying on molecular biomarkers to evaluate degradation potential *in situ*.

Objectives

The BioDissPOL (Microbial biomarkers: applicability for diagnostic and monitoring of polluted sites) project, funded by ADEME, addresses how dissipation of chloroethenes is linked with functional and taxonomic microbial markers, with the aim to develop a widely-accepted framework to monitor natural attenuation at contaminated sites.

Methods

We collected groundwater biannually from 12 piezometers in a multi-polluted site to *(i)* assess degradation conditions based on physico-chemical parameters and *(ii)* quantify the extent of degradation using stable isotope analysis (CSIA), in order to *(iii)* link this information with copy numbers of functional genes and changes in microbial community profiles using next generation sequencing (Illumina MiSeq).

Conclusions

Significant changes in carbon isotope composition accompanied by decrease in perchloroethylene and increase in dichloroethene and vinyl chloride concentrations throughout the plume, demonstrated the occurrence of *in situ* biodegradation. Taxonomic and functional analyses identified the presence of dehalorespiring genera with functional genes involved in biodegradation of chloroethenes in groundwater. Taken together, our results highlight that preliminary evaluation of chloroethenes biodegradation provides valuable information to bioremediation strategies at contaminated industrial sites.

FEMS7-1629
Environmental Microbiology/Microbial Ecology /Microbial Communities

INVESTIGATION IN THE POTENTIAL ROLES OF VISCOSINAMIDES PRODUCED BY P. FLUORESCENS

S. Depayras[1], A. Merieau[1], M. Barreau[1], T. Kondakova[1,2], C. Barbey[1,3], M. Gallique[1], M. Feuilloley[1], N. Orange[1], C. Duclairoir-Poc[1]
[1]University of Rouen Normandy, Laboratory of Microbiology Signals and Microenvironment, Evreux, France
[2]University of Illinois, Cronan Lab- Department of Microbiology, Urbana, USA
[3]North committee, Seeds Innovation Protection Research Environment, Achicourt, France

Backgrounds

Biosurfactants are amphiphilic compounds exhibiting low toxicity and high biodegradability. Produced by a wide range of microorganisms, they are nowadays commonly used in pharmaceutical, food cosmetic and bioremediation industries. However few studies focus on the potential role of cyclolipopeptides (CLPs) unlike well-known rhamnolipids.

Objectives

We investigated here new potential roles of viscosinamides, CLPs produced by a clinical *P. fluorescens* strain MFN1032. We focused on their implication in colonization, virulence and cell-to-cell communication.

Methods

CLPs of MFN1032 were isolated and identified by RP-HPLC-MS. Then MIC and cytotoxicity assays were performed respectively on cutaneous strains and pulmonary cell line (A549). Meanwhile adhesion and biofilm development of MFN1032 were quantified by confocal microscopy. Moreover quorum sensing tests were realized using two biosensor strains detecting N-AcylHomoserine Lactones (NAHLs) production.

Conclusions

P. fluorescens MFN1032 produced abundantly viscosinamide A and poorly other viscosinamides. CLPs contributed in embedding the matrix but also take part in the escape of asocial cells out of microcolonies by motility alteration. Cell free supernatant promoted the lysis of pneumocytes emphasing the potential cytotoxicity of CLPs or other exoproducts. CLP extracts induced an alteration of the growth of Gram positive bacteria. However MFN1032 did not seem to produce NAHLs. Interestingly MFN1032 was able to communicate, when placed in proximity with biosensors, suggesting a potential new way of cell-to-cell communication. The chemical similarities between NAHL and viscosinamides and their low diffusibility let us suspect the potential implication of CLPs in this phenomenon.

FEMS7-1651
Environmental Microbiology/Microbial Ecology /Microbial Communities

IMPACT OF GASEOUS NO2 ON P. FLUORESCENS STRAIN IN THE MEMBRANE ADAPTATION AND VIRULENCE

T. Kondakova[1,2], S. Depayras[1], N. Merlet-Machour[3], H.J. Heipieper[4], C. Catovic[1], M. Barreau[1], M. Gallique[1], M. Feuilloley[1], N. Orange[1], C. Duclairoir-Poc[1]
[1]University of Rouen Normandy, Laboratory of Microbiology Signals and Microenvironment, Evreux, France
[2]University of Illinois, Cronan Lab- Department of Microbiology, Urbana, USA
[3]University of Rouen Normandy, COBRA UMR 6014, Evreux, France
[4]Helmholtz Centre for Environmental Research, Department of Environmental Biotechnology, Leipzig, Germany

Backgrounds

Nowadays air pollution is clearly increasing due to anthropogenic activity despite more drastic regulations. Among all air pollutants, nitrogen oxides, such as NO and NO_2, are predominant. Such compounds exhibit direct toxic effects on humans especially on lungs. However microorganisms are also exposed to them but their synergy with microorganisms on microbial virulence is still not stated.

Objectives

We studied the impact of one major air pollutant, the nitrogen dioxide, on an environmental strain of *P. fluorescens* MFA76a, mainly in terms of its adaptability and virulence factors.

Methods

MFAF76a was exposed in a home-made exposure system at 45ppm of NO_2. Afterwards growth kinetics, MIC assays and cultivability were observed as the membrane permeability. Meanwhile HPTLC/MALDI-TOF MS Imaging characterized the membrane lipids. Finally transcriptomic studies by RT-qPCR were performed on NOx detoxification gene and RND class efflux pump.

Conclusions

An increasing of membrane permeability and a decrease of one log in cultivability were observed after a two hour exposure at 45ppm of NO_2. Interestingly a lipidomic study confirmed the alteration in membrane lipid composition. Following exposure an glycerophospholipid, still uncharacterized, disappeared. However transcriptomic studies indicated an up-regulation in the expression of genes implicated in NOx detoxification (*hmp*) and RND class efflux pump (*mexEF-oprN* and *mexXY)*, also implicated in antibioresistance as confirmed by MIC assays.

FEMS7-2134
Environmental Microbiology/Microbial Ecology /Microbial Communities

MONITORING OF PHYSICOCHEMICAL AND MICROBIOLOGICAL FEATURES OF AGRICULTURAL AND FOREST SOILS AFTER AN OIL PIPELINE IMPLANTATION

M.G. Bonomo[1], L. Calabrone[1], K. Di Tomaso[1,2], S. Sasso[1], L. Scrano[3], S.A. Bufo[1], G. Salzano[1]
[1]Università degli Studi della Basilicata, Sciences, Potenza, Italy
[2]Università degli Studi della Basilicata, Ph.D school in Applied Biology and Environmental Safeguard, Potenza, Italy
[3]Università degli Studi della Basilicata,
European and Mediterranean Cultures. Architecture- Environment and Cultural Heritage, Matera, Italy

Backgrounds

The biodiversity of soil, due to variety of chemical and metabolic processes, has an important role in maintaining ecosystems in a functionally efficient state. Changes and dynamics of soil ecosystem allow a stabilization of the functional interrelationships between the various micro-organisms, the plants and the animal community.

Objectives

In this study physical, chemical and microbiological characteristics of the agricultural and forest soil areas were determined to assess dynamics and the evolution processes induced by environmental restoration procedures after an oil pipeline implantation.

Methods

Soil samples were collected from forest and agricultural areas in a Southern Italy farm, and analyzed for specific parameters and biological indicators, such as total biomass-C, organic-C, respirometric tests and microbial community composition. Soils were sampled ante-opera to use as control and after one and two years from oil pipeline implantation.
Agricultural and forest area samples showed an interesting diversification of the specific parameters for each sampling site, with significant changes of textural fractions, high values of pH and a remarkable bio-stimulation of germination and a strong enrichment of edaphic fauna.
The microbial community composition proved the presence of particular species, usually current in contaminated/strongly disturbed soils, involved in bioremediation/biodegradation natural process to restore optimal conditions.

Conclusions

The elevated intra-specific and functional biodiversity underlined the soil capacity to tolerate and withstand the disturbances with the presence and the adaptation of bacterial species able to perform certain functions in alteration conditions.

FEMS7-0292
Environmental Microbiology/Microbial Ecology /Microbial Communities

HPTLC CHROMATOGRAPHY AS IDEAL TOOL FOR SEPARATION A MIXTURE OF BACILLUS LIPOPEPTIDE EXTRACTS IN SITU

I. Dimkić[1], P. Ristivojević[2], T. Berić[1], T. Janakiev[1], I. Nikolić[1], Đ. Fira[3], S. Stanković[1]
[1]Faculty of Biology - University of Belgrade, Microbiology, Belgrade, Serbia
[2]Innovative Centre of Faculty of Chemistry Ltd.- University of Belgrade, Analytical chemistry, Belgrade, Serbia
[3]Faculty of Biology - University of Belgrade, Biochemistry and Molecular Biology, Belgrade, Serbia

Backgrounds

Bacillus species produce a wide array of biologically active molecules, including nonribosomaly synthesized lipopeptides. Bioautography is a valuable tool for the identification of bio-active compounds from complex mixtures.

Objectives

Detection of antimicrobials production by *Bacillus* species is important in determining its capability to be a good biocontrol agent.

Methods

Antimicrobial activity of lipopeptide extracts of five *Bacillus* spp. strains (SS-10.7, SS-12.6, SS-13.1, SS-38.4 and SS-27.2) was tested against *Pseudomonas syringae* pv. *aptata* from sugar beet, *Xanthomonas arboricola* pv. *juglandis* from walnut tree, and *Listeria monocytogenes*. HPTLC chromatography was used for simultaneous separation of complex mixtures of lipopeptide extracts and for determination of antimicrobial activity of separated components by agar overlay method. Standard compounds of iturin A and surfactin were used as positive controls.

Conclusions

For the inhibition of *Xanthomonas* and *Pseudomonas* iturin analog was responsible. The growth inhibition was not observed for surfactin standard. SS-12.6, SS-13.1 and SS-38.4 extracts exhibited the high inhibition zones, while extracts of SS-10.7 and SS-27.2 showed weak effect against all tested pathogens. The highest inhibition was recorded for extract of SS-12.6 within R_F values of 0.20 and 0.28 indicating synergism of several analogues of iturin. HPTLC bioautography identified the active components from a mixture of lipopeptide extracts in a very effective manner, *in situ* proving its potential for use in direct detection and determination of antimicrobials.

FEMS7-0189
Environmental Microbiology/Microbial Ecology /Microbial Communities

CORRELATIONS BETWEEN GUT MICROBIOTA, PHYSIOLOGY, XENOBIOTICS INTAKE AND ANTIMICROBIAL RESISTANCE GENES IN OBESE, OVERWEIGHT AND EUTROPHIC INDIVIDUALS.

V.L. Silva[1], S. Marjorie Raquel Anariba[1], T.O. Paula[1], F.M. Borges[1], A.B. Ferreira-Machado[1], J.A. Resende[2], C.G. Diniz[1]
[1]Universidade Federal de Juiz de Fora, Departamento de Parasitologia- Microbiologia e Imunologia, Juiz de Fora, Brazil
[2]Universidade Federal do Espírito Santo, Departamento de Farmácia e Nutrição, Alegre, Brazil

Backgrounds

As obesity is related to the gut microbiota (GM), studies on microbial ecology in obese individuals are needed. GM is considered an important reservoir of antimicrobial resistance genes (ARG). Little is known on GM and ARGs in obesity, considering the altered physiology and xenobiotics intake.

Objectives

This study aimed in a comparative screening and correlations between gut microbiota, physiology, xenobiotics intake and AGRs in obese, overweight and eutrophic individuals.

Methods

Fecal metagenome of 72 individuals classified according to their body mass index (BMI) was used as template for PCR targeting 59 different ARGs and evaluation of bacterial density by FISH. Anthropometric/nutritional characteristics, and xenobiotics intake were recorded for correlation analysis.

Conclusions

Among 27 ARGs detected, a cluster of 17 was observed in all groups suggesting a common core. In general, ARGs were observed mostly within obeses, followed by overweight and eutrophic individuals. Bacterial groups showed to be increased, in terms of relative density, among obese individuals. The ratio between ARGs and bacterial groups may suggest that AGRs were more related to enterobacteria. Positive correlations were observed between ARGs and BMI, calories and xenobiotics intake (especially use of sweeteners). Overall the results are in agreement with observations that GM is altered in obesity. However the altered physiology in obese individuals seems to be also associated to the increased xenobiotics intake and may interfere with GM towards antimicrobial resistance, as observed by the fecal metagenome and ARGs screening. Overweight individuals behave as an intermediary category between eutrophic and obese. Support: FAPEMIG, CNPQ, CAPES, PPGCBIO/UFJF.

FEMS7-3137
Environmental Microbiology/Microbial Ecology /Microbial Communities

SEQUENCE AND ACTIVITY-BASED METAGENOMICS APPROACHES TO DISCOVER INDUSTRIALLY-RELEVANT ENZYMES

M.A. Distaso[1], S. Wright[2], C. Coscolin[3], V. Tverezovskiy[2], M. Ferrer[3], T. Hai[1], P. Golyshin[1]
[1]Bangor University, School of Biological Sciences, Bangor, United Kingdom
[2]Bangor University, Biocomposites Centre, Bangor, United Kingdom
[3]Consejo Superior de Investigaciones Cientificas, Institute of Catalysis, Madrid, Spain

Backgrounds

In the modern industrial era the demand for suitable enzymatic biocatalysts to use as greener alternative to chemical synthesis is crucial. Enzymatic routes become a successful application when they contribute to an overall cost reduction, an increased product yield and lower volumes of wastewater streams and by-products. Uncultured microorganisms are an exceptional resource of powerful biocatalysts capable of performing a wide range of reactions and of accepting extensive collections of complex molecules as substrates.

Objectives

We report on the application of metagenomic screening methods for the recovery of biocatalysts of industrial interest to use in enzymatic processes.
This work was specifically aiming at finding (1) a methylation reaction for the enzymatic pathway to produce a potent weed control compound

Methods

The soil was enriched with lipophilic compounds resembling the target substrate for the enzymatic conversion. Consequently, total DNA was recovered and subjected to *de novo* sequencing and in *silico* annotation, which has yielded few candidate genes.

Conclusions

One of them from a representative of the phylum *Actinobacteria* appeared an efficient SAM-*O*-methyltransferase able to accommodate and methylate the moiety of our substrate molecule. Preliminary tests showed up to 30% of substrate conversion in a not yet optimized reaction environment.
Contextually, (2) functional screening of the fosmid libraries established from the same source identified two highly promiscuous lipases capable of hydrolysing the broader range of ester substrates than the most-active industrial prototypes.

Characterization and site directed mutagenesis studies are ongoing, with the potential to make these enzymes extremely useful in a number of biotechnological applications.

FEMS7-1806
Environmental Microbiology/Microbial Ecology /Microbial Communities

ISOLATION AND CHARACTERIZATION OF HYDROCARBONOCLASTIC BACTERIA FROM ALGERIAN COAST

N. Djahnit[1], S. Chernai-hamdi[1], B. Hamdi[1], B. China[2], V. Catania[3], S. Cappello[4], P. Quatrini[3]
[1]Ecole Nationale des Sciences de la Mer et l'Aménagement du Littoral,
Laboratoire de conservation et de valorisation des ressources marines, Algiers, Algeria
[2]Institut Scientifique de santé publique, Institut Scientifique de santé publique, Bruxelles, Belgium
[3]University of Palermo,
Dept. of Biological- Chemical and Pharmaceutical Sciences and Technologies STEBICEF, PALERMO, Italy
[4]Istituto per l'Ambiente Marino Costiero IAMC, Istituto per l'Ambiente Marino Costiero IAMC, MESSINA, Italy

Backgrounds

The contamination of marine environments by hydrocarbons represents a global concern with consequences on ecosystems and human health. The removal of HC by physical and/or chemical methods is expensive and disrespectful of the environment. The use of HC degrading bacteria is a good alternative for environmental remediation (bioremediation).

Objectives

The Algerian coastline is exposed to several types of pollutions, including hydrocarbons. The aim was to explore the bioremediation potential of its contaminated harbors for the first time.

Methods

To identify the hydrocarbon degrading bacteria seawater samples were collected in Sidi fredj harbor (Algeria) and used to enrichment cultures on mineral medium supplemented with Algerian light crude oil. The isolates were characterized by biochemical tests and Gram straining. The taxonomic identification of the isolates was performed by ARDRA and sequencing of the 16S rRNA gene. The ability to grow on hydrocarbons and degradation abilities were analysed by GC-FID together with their emulsification capacity. The key catabolic genes involved in HC degradation were detected by PCR.

Conclusions

About twenty isolates were assigned to known hydrocarbonoclastic genera (*Alcanivorax, Halomonas, Marinobacter*) and generalist HC degraders (*Pseudomonas*), but also to *Bizionia* (class Flavobacteriia). The isolates grow on *n*-alkanes (C_{16} to $C_{24,}$ *Alcanivorax, Halomonas*) toluene and benzene (*Marinobacter*) and a show average oil emulsification capacity. The PCR and sequencing of catabolic genes confirmed the presence of alkane hydroxylase genes and degradation abilities analysis is in progress. Further characterization will allow us to identify the most interesting strains to perform bioremediation.

FEMS7-2688
Environmental Microbiology/Microbial Ecology /Microbial Communities

REMOVAL OF ENVIRONMENTALLY-PERSISTENT, AND COMMENSAL, E. COLI STRAINS FROM DRINKING WATER BY SLOW SAND FITLER: PERFORMANCE AND MICROBIAL ECOLOGY

O. Domingo Fernandez[1], *G. Collins*[1]
[1]*National University of Ireland- Galway, Microbiology, Galway, Ireland*

Backgrounds

Slow Sand Filters (SSFs) provide a cost-effective and energy-efficient option for drinking water production. Decontamination is achieved by biological processes in SSF biofilm. SSFs also provide a fascinating environment to study the microbial ecology underpinning pathogen removal mechanisms. At the same time, recent studies detected *E. coli* strains persisting in cold soils, putting into question whether *E. coli* is a reliable indicator organism of faecal contamination.

Objectives

In this study, the objectives were to assess the *E. coli* retention capacity of SSFs and compare retention rates of an environmentally-persistent and a commensal *E. coli* strain. The impact of sporadic faecal contaminations on the structure of bacterial and protozoan communities was also investigated.

Methods

Nine laboratory-scale SSFs were operated for 210 days and several contamination events were simulated in six of the filters. Each event comprised dosing the influent with *E. coli* (10^8 cfu/100ml) and monitoring the concentration in the effluent to determine SSF rentention of the coliforms. No contamination spikes were applied to the three control SSFs. *E. coli*, total bacteria and total protozoa were quantified in sand biofilm samples by targeting *rodA,* and 16S and 18S rRNA, genes, respectively, in qPCR assays. Bacterial and protozoan community structure and diversity were determined using high-throughput sequencing of 16S and 18S rRNA genes.

Conclusions

Excellent retention was achieved, and retention rates of the environmentally-persistent and commensal strains were similar. Certain protozoan families were more abundant after *E. coli* spikes. Overall, faecal contamination events played a major role in determining SSF microbial community structure.

FEMS7-2800
Environmental Microbiology/Microbial Ecology /Microbial Communities

PROKARYOTIC ASSESSMENT IN GEOTHERMAL AND VOLCANIC ZONES IN ARGENTINA: METAL AND METALLOID RESISTANCES AND BIOTECHNOLOGICAL APPLICATIONS

E. Donati[1], F. Massello[1], A. Lima[1], A.B. Segretin[1], C. Castro[1], G. Willis Poratti[1], M. Hipperdinger[1], M.S. Urbieta[1]
[1]CINDEFI, Quimica, La Plata, Argentina

Backgrounds

Assessing microbial communities in extreme environments such as volcanic/geothermal zones, allows for the search of novel extremophilic microorganisms that can be of use in the development or improvement of biotechnological processes. Most microbial communities developed in such harsh environments have different heavy metal resistance strategies of importance in some technological applications as biomining but also in the bioremediation of metal(loid) polluted environments.

Objectives

Our aim was to investigate the microbial diversity of Copahue geothermal system with temperatures up to 90°C and pH values from 2 to 7 under aerobic and anaerobic conditions.

Methods

Three different approaches were used, enrichment to isolate autochthonous microorganisms able to grow in the presence of high metal(loid) concentrations, and two culture independent alternatives (amplification and sequencing of the complete 16S rRNA gene of the entire community and fluorescent *in situ* hybridization). In addition, such communities were used in biotechnological applications related to heavy metal bioremediation .

Conclusions

Bacteria (acidophilic species related to AMD) dominated the acidic areas of moderate temperature, while archaea prevail in high temperature ponds. In the water samples, the dominant prokaryotes were chemolithoautotrophic or mixotrophic, mainly sulfur oxidizers while in microbial biofilms photosynthetic species were the most important primary producers. In the case of the acidic river, Rio Agrio, also mainly sulfur oxidizing bacteria and archaea were found although some iron oxidizing microorganisms were also detected. Some microbial communities isolated showed the ability to precipitate metals even at very low pH values and to reduce hexavalent chromium to the less toxic trivalent chromium.

FEMS7-2809
Environmental Microbiology/Microbial Ecology /Microbial Communities

CULTIVATION-DEPENDENT AND INDEPENDENT APPROACHES FOR DETERMINING PROKARYOTIC DIVERSITY IN ACID MINE DRAINAGE

E. Donati[1], L. Benitez[1], A. Muñoz[1], C. Bernardelli[1], J. Plaza Cazón[1], M.S. Urbieta[1]
[1]CINDEFI, Quimica, La Plata, Argentina

Backgrounds

Sulfide ores usually rich in heavy metals are a potential source for acid mine/rock drainage (AMD/ARD) which can cause severe contamination of surface and groundwater, also impacting on soils and plants constituting a serious environmental problem. AMD/ARD are produced when metal sulfides –mainly pyrite- are exposed to air and water. Although the process can occur under abiotic conditions, microbial activity can highly increase the rate of acid drainage generation. In order to prevent and/or remediate AMD/ARD, the native microbial communities must be assessed. It is widely accepted that culture-independent methods have many advantages on the traditional isolation techniques for microbial community analysis. However, for determining the role and the impact of indigenous communities on certain processes –like AMD/ARD generation- the contribution of cultivation approaches cannot be discarded.

Objectives

The aim of this work was to assess the prokaryotic diversity on the acid drainages of the Pan de Azúcar mine (Jujuy, Argentina) and Amarillo River (Famatina Belt, La Rioja, Argentina).

Methods

Denaturating gel gradient electrophoresis (DGGE) and metagenomic were used to determine the structure of the microbial communities. Cultivation techniques were also used.

Conclusions

Based on such results cultivation techniques were used to isolate microorganisms, although only a limited number of the predicted species were obtained. In addition, the isolates were tested on samples of tailings from Pan de Azucar mine and on water samples from Amarillo River. The results of our study show a decisive role of the species isolated on the AMD/ARD generation and on the geochemical characteristics of both acid drainages.

FEMS7-1211
Environmental Microbiology/Microbial Ecology /Microbial Communities

PROMOTING BIOFILM DEVELOPMENT OF CATALYTIC BACTERIA FOR BOOSTING BIODEGRADATION OF DBT

P. Dorado-Morales[1], B. García[1], I. Martínez[2], E. Díaz[2], I. Lasa[1], C. Solano[1]
[1]Centro de Investigación Biomédica Navarrabiomed/ UPNA, Laboratory of Microbial Pathogenesis, Pamplona, Spain
[2]Centro de Investigaciones Biológicas. CSIC, Laboratory of environmental microbiology, Madrid, Spain

Backgrounds

Biofilms are structured communities of microorganisms that grow adhered to inert or living surfaces due to a self-produced exopolymeric matrix in which they are encased. Inside the biofilm, bacteria exhibit a superior tolerance to physicochemical insults and harsh reaction conditions. These properties that might have deleterious consequences in clinical settings, are of great interest in industrial and environmental biotechnology, such as in the field of biocatalysis of toxic compounds.

Objectives

The aim of this study is to evaluate whether genetically engineered bacteria forced to grow as biofilm communities, through either the production of a heterologous polysaccharide or the overproduction of their own exopolysaccharides, show improved metabolic capabilities. As a proof of concept, we have used the gram-positive bacterium *Rhodococcus erythropolis* IGTS8 that harbors the *dszABCD* genes, through which the model compound dibenzothiophene (DBT) is transformed into the sulfur-free 2-hydroxybiphenyl (2HBP) molecule. *Rhodococcus erythropolis* engineered bacteria that grow inside a biofilm might provide an improved biotechnological strategy for the removal of the recalcitrant sulfur of aromatic heterocycles present in fuels.

Methods

Genetic engineering of a *Rhodococcus erythropolis* strain able to desulfurinate DBT in order to promote biofilm development.

Genetic engineering of *dsz* cassettes to avoid bottlenecks in the conversion of DBT into 2HBP.

Analysis of DBT desulfurization efficiency through the quantification of DBT and 2HBP by HPLC.

Conclusions

Preliminary results show that genetic programming of *Rhodococcus* bacteria to form a biofilm through the overproduction of its own exopolysaccharides leads to an increase in the bioconversion of DBT into 2HBP.

FEMS7-1656
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE EFFECTS OF GROWTH CONDITIONS AND SECONDARY ENVIRONMENTAL STRESSES ON THE RESPONSE OF LISTERIA MONOCYTOGENES TO VISIBLE LIGHT

A. Dorey[1], *B. O'Donoghue*[1], *K. NicAogáin*[1], *C. O'Byrne*[1]
[1]*Bacterial Stress Response Group, Microbiology, Galway, Ireland*

Backgrounds

Research investigating the response of *L. monocytogenes* to visible light has identified the Lmo0799 protein in the sensing of visible light, and the activation of the SigB signalling cascade. Triggering of the σ^B signalling cascade leads to the transcription of the general stress response gene regulon via the activation of σ^B. Growth of *L. monocytogenes* in alternating periods of light and dark results in a ringed colony morphology, preventable by the deletion of *sigB* or *lmo0799* from the genome. The inhibitory effect of visible light on *L. monocytogenes* is due to accumulation of reactive oxygen species, and exposure to visible light induces a protective response against a challenge by ROS.

Objectives

The aim of our study is to elucidate the role of σ^B in the resistance of *L. monocytogenes* to visible light using transcriptomic and phenotypic experiments.

Methods

L. monocytogenes wild-type and Δ*sigB* deletion mutant cultured at 30°C or 37°C were challenged with visible light at exponential and stationery growth phases. Cells were pre-exposed to sub-lethal doses of environmental stresses to investigate cross-resistance between visible light and alternative stresses. RT-PCR targeted at σ^B-dependent genes was used to determine the optimum dose and exposure time for the activation of σ^B by visible light.

Conclusions

Our research shows that growth phase and temperature affect susceptibility of *L. monocytogenes* to visible light, with cells being more susceptible at exponential compared to stationery phase and at 37°C compared to 30°C, and the role of σ^B in the protective response. Some environmental stresses convey resistance to visible light.

FEMS7-2900
Environmental Microbiology/Microbial Ecology /Microbial Communities

ANTIBIOFILM ACTIVITIES OF MULTIPURPOSE SOLUTIONS AGAINST SOFT CONTACT LENS BIOFILM MODELS

S. Dosler[1], *M. Tuysuz*[1]
[1]*Istanbul University- Faculty of Pharmacy, Pharmaceutical Mirobiology, Istanbul, Turkey*

Backgrounds

Contact lenses are important bio-medical substances with not only the correction of some eye defects but also the cosmetic characteristics. Especially the formation of biofilms with adherent bacteria is very important menace for whole eye health. Especially multi drug resistant *Pseudomonas aeruginosa* or methicillin resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* strains which are the common pathogen bacteria and yeast, respectively, in eye infections.

Objectives

Because of their increasing resistance to antibiotics, the treatment of them is very difficult today, especially when they are being in biofilms. In this study, we investigated the in vitro activities of some multi-purpose lens solutions. (Bio-true, renuu, opti-free and all-in-one) and antibiotics (gentamycin, tobramycin and ciprofloxacin) against *P. aeruginosa*, MRSA, and *C. albicans* biofilms on two kind of soft contact lenses with time killing curve method at 6, 24 and 48 hours.

Methods

In vitro activities of iMulti-purpose lens solutions (Bio-true, renuu, opti-free and all-in-one) and antibiotics (gentamycin, tobramycin and ciprofloxacin) against *P. aeruginosa*, MRSA, and *C. albicans* biofilms on two kind of soft contact lenses were tested with time killing curve method at 6, 24 and 48 hours.

Conclusions

The most active multipurpose solution against *P. aeruginosa* and MRSA biofilms at 24 hours was opti-free fallowed by the bio-true and renuu, against *C. albicans* biofilms at 48 hours was renuu fallowed by the opti-free and bio-true. In addition, all studied antibiotics were active against *P. aeruginosa* and MRSA biofilms on soft contact lenses at 24 hours. According to our results, anti-biofilm activities of multipurpose lens solutions were various depending the chemical ingredients and contact time.

FEMS7-1054
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROBIAL DYNAMICS AND SUCCESSION IN DRINKING WATER DISTRIBUTION SYSTEMS

I. Douterelo[1], *K. Fish*[1], *J. Boxall*[1]
[1]*Pennine Water Group- The University of Sheffield, Civil and Structural Engineering, Sheffield, United Kingdom*

Backgrounds

Biofilms formed on inner-pipe surfaces of drinking water distribution systems (DWDS) can affect the quality and safety of drinking water. Biofilm formation is a successional process beginning when free-living microorganisms attach to pipe surfaces, growing whilst modifying the pipe wall, generating extracellular polymeric substances that facilitate the incorporation of other microorganisms into the biofilm. The community characteristics for such succession are largely unstudied, particularly for representative DWDS conditions and mixed microbial communities.

Objectives

To study the initial succession of water infrastructure biofilm communities over three months of development under conditions fully representative of operational DWDS.

Methods

Biofilms were developed and monitored over a three month period in a full-scale experimental pipe loop facility at 16°C (average UK summer water temperature). Hydraulic conditions were controlled to simulate demand driven daily patterns. Biofilms were sampled using coupons removed at regular time intervals. Water physico-chemical parameters were analysed using discrete sampling. Bacterial and fungal communities were characterised using Illumina sequencing of 16S and 18S rRNA genes, respectively.

Conclusions

Bacteria were found to be the predominant taxa compared to fungi within the DWDS biofilms. Fungal communities did not experience substantial temporal changes. However, bacterial communities were observed to increase in complexity over the three-month period. Indicative of successional colonisation of the pipeline from primary and secondary colonisers to a more complex, maturing biofilm. We can conclude that biofilm formation is a highly dynamic process occurring in relative short-time periods, dominated by bacteria and influenced by internal and external factors.

FEMS7-2103
Environmental Microbiology/Microbial Ecology /Microbial Communities

HIGH THROUGHPUT SEQUENCING OF FULL-LENGTH SSU RRNA SEQUENCES FROM COMPLEX MICROBIAL COMMUNITIES WITHOUT PRIMER BIAS AND HOW IT AFFECTS OUR ABILITY TO STUDY MICROBIAL ECOLOGY

M.S. Dueholm[1], S.M. Karst[1], S.J. McIlroy[1], R.H. Kirkegaard[1], P.H. Nielsen[1], M. Albertsen[1]
[1]Aalborg University, Department of Chemistry and Biosciences, Aalborg East, Denmark

Backgrounds

The small subunit (SSU) ribosomal RNA (rRNA) genes have been used to study microbial diversity and evolution for the last 30 years. Today, databases containing full-length SSU rRNA reference sequences remain fundamental for many core tools in microbial ecology, such as community profiling using 16S/18S amplicon sequencing and *in-situ* studies based on fluorescence *in situ* hybridization microscopy. The quality of the data produced relies heavily on the reference databases used, and it is widely recognized that the current databases are underpopulated, ecosystem skewed, and subject to primer bias. This is a consequence of the present low-throughput, primer-based methods used to generate the sequences.

Objectives

We want to develop a method that allows high-throughput sequencing of full-length SSU genes without the use of conventional primers. Furthermore, we will examine how the access to comprehensive ecosystem specific SSU databases affect our ability to study microbial ecology.

Methods

We have developed a method which combines reverse transcription of environmental full-length SSU rRNA sequences and Illumina based synthetic long-read sequencing to obtain high quality, full-length SSU rRNA sequences in a high throughput manner.

Conclusions

In our initial trials, we obtained more than 40,000 high quality SSU rRNA sequences (> 1200 bp) representing all domains of life from five different ecosystems (human gut, soil, fresh water, anaerobic digestion and activated sludge), showing the general application and the throughput of the method. Here we describe how the method works and demonstrate how the access to comprehensive ecosystem specific SSU databases affect our ability to study microbial ecology.

FEMS7-1011
Environmental Microbiology/Microbial Ecology /Microbial Communities

SOIL MICROBIAL DIVERSITY: A COMPARISON OF METAGENOMICS AND CULTUROMICS APPROACHES

C. Dumolin[1], *P. Vandamme*[1]
[1]*Ghent University, Biochemistry and Microbiology, Gent, Belgium*

Backgrounds

Novel insights in the tree of life revealed staggering numbers of uncultivated bacterial lineages. Harnessing this novel biotechnological potential requires prior cultivation. Although metagenomics studies are considered the gold standard for analyzing microbial diversity, large scale isolation and identification campaigns, i.e. ”culturomics “, may reveal organisms that went undetected via metagenomics. A combined approach, is therefore paramount to determine the microbial diversity and to exploit its biological functionalities.

Objectives

The aim of the present study was to determine the microbial diversity in an old-growth forest soil sample using both metagenomics and culturomics.

Methods

A plain soil sample of the Aelmoeseneie forest (Gontrode, Belgium) was collected. For metagenomic analyses, DNA was extracted using the Mobio Powersoil kit. Total DNA was sequenced on the Illumina HiSeq platform. The cultivable microbial diversity of the same soil sample is studied via an in-house developed culturomics pipeline, based on the automated picking of isolates and dereplication via MALDI-TOF MS. We aim to isolate and identify 25,000 bacterial isolates.

Conclusions

Shotgun metagenomic sequencing yielded >237,000 reads, correlating to a bacterial richness of >800 species. This diversity is dominated by *Proteobacteria* (55%), *Actinobacteria* (35%) and *Acidobacteria* (3%). *Proteobacteria* are mainly represented by *Alphaproteobacteria* with *Rhizobiales* (78%) and *Rhodospirillales* (7%) as main orders. *Actinobacteria* are dominated by the *Corynebacteriales* order (31%); interestingly, 8% of the total DNA sampled represented *Mycobacterium* reads. The culturomics analysis is ongoing; in summer 2017 half of the culturomics data, i.e. referring to the isolation and identification of about 12,500 isolates, will be available and presented.

FEMS7-1465
Environmental Microbiology/Microbial Ecology /Microbial Communities

ANAMMOX FOR MAINSTREAM NITROGEN REMOVAL FROM WASTEWATER

M. Duran[1], *D. Foster*[1], *S. Muller*[1]
[1]*Villanova University, Civil and Environmental Engineering, Villanova, USA*

Backgrounds

Anaerobic ammonium oxidation (anammox), is the biochemical process whereby bacteria oxidize NH_4^+ to N_2 using NH_4^+ as the electron donor and NO_2^- as the electron acceptor. Anammox bacteria, discovered in the mid-1990s,[1] are fairly common in oxygen-limited natural habitats[2,3] and allow one-step conversion of NH_4^+ to N_2, thereby abbreviating the nitrogen cycle. Anammox offers a transformative alternative for NH_4^+ removal from wastewaters, as it requires 75% less oxygen than the traditional nitrification/denitrification process. In addition, it reduces emissions of the greenhouse gas N_2O by 90%.[4,5,6]

Although anammox has been applied for the treatment of warm and high-strength wastewaters [24,25] there are significant challenges preventing its wider application for NH_4^+ removal from wastewaters. A recent survey by Lackner *et al.* (2014) points out the difficulty of developing and retaining sufficient anammox biomass, due to the anammox bacteria's unusually slow growth rates.[7] In addition, current anammox applications require partial denitritation in order to supply NO_2^- per the anammox reaction. This has limited the current anammox technologies to sidestream NH_4^+ removal where approximately 500 mg-N/L is required.

Objectives

This objective of this study was to cultivate anammox suitable for mainstream NH_4^+ removal from wastewaters.

Methods

A bench-scale bioreactor (15 L effective volume) was operated for over one year with the anammox culture, obtained from a full-scale DEMON® reactor, growing on NH_4^+ concentrations typically seen in municipal wastewater, 50 mg-N/L.

Conclusions

The results indicate that the selected anammox culture consistently achieved over 90% NH_4^+ removal, showing that mainstream NH_4^+ from wastewater is feasible.

FEMS7-2541
Environmental Microbiology/Microbial Ecology /Microbial Communities

AROMATIC RING-HYDROXYLATING OXYGENASE-ENCODING GENES OF THE HYDROCARBONOCLASTIC MARINE BACTERIUM ALCALIGENES SP. QD168

R. Durán[1,2], F. Salvà-Serra[3,4], B. Barra[1,2], E. Moore[3,4], M. Seeger[1,2]
[1]Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química- Universidad Técnica Federico Santa María, Valparaiso, Chile
[2]Center of Biotechnology, Universidad Tecnica Federico Santa Maria, Valparaiso, Chile
[3]Culture Collection University of Gothenburg CCUG, University of Gothenburg, Gothenburg, Sweden
[4]Department of Infectious Diseases- Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Backgrounds

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous, toxic and recalcitrant environmental pollutants. Microbial degradation has been applied to remove these pollutants. The aromatic ring-opening is a key enzymatic step catalyzed by multicomponent aromatic-ring-hydroxylating oxygenases (ARHOs).

Objectives

The aim of this study was the identification of ARHO-encoding genes in the marine *Alcaligenes* sp. QD168 genome.

Methods

Bacterial strain QD168 was isolated after an oil spill from marine sediment of Quintero Bay, Central Chile. QD168 cells growth on various PAHs as sole carbon source was assessed. Strain QD168 genome was sequenced, assembled and automatically annotated by RAST, PROKKA and further analyzed through a curated search to predict ARHO α-subunits. A phylogenetic tree was constructed using amino acid sequences of α-subunit terminal oxygenases**.**

Conclusions

QD168 cells were able to grow on fluorene, naphthalene and phenanthrene as sole carbon sources. After the assembly, we obtained a 4.28 Mb draft genome (GC-content 56.4%). More than 30 α-subunit terminal oxygenase-encoding genes were identified. The bayesian tree clustered QD168 α-subunits with reference ARHO genes, identifying the presence of different terminal oxygenase classes. In conclusion, this study revealed the existence of a wide range of terminal oxygenase-encoding genes in *Alcaligenes* sp. QD168, reflecting its potential for bioremediation of aromatic compounds.

Acknowledgments: MSc CONICYT-PCHA/Magister Nacional/2016-22162307 (RD) & PhD CONICYT-PCHA/Doctorado Nacional/2013-21130742 (BB) fellowships, FONDECYT 1151174 (MS) and Swedish Västra Götaland Region (ALFGBG-437221) (FS-S,EM) grants.

FEMS7-3143
Environmental Microbiology/Microbial Ecology /Microbial Communities

ARSENIC EFFECT UPON LIPID COMPOSITION IN A NOVEL HALOPHILE WITH POTENTIAL BIOREMEDIATION APPLICATIONS.

V. Durán-Toro[1], M. Kellermann[2], K.U. Hinrichs[3], S. Bühring[1]
[1]MARUM, Hydrothermal Geomicrobiology, Bremen, Germany
[2]Institut für Chemie und Biologie des Meeres, Umweltbiochemie, Wilhelmshaven, Germany
[3]University of Bremen, MARUM, Bremen, Germany

Backgrounds

Bioremediation of arsenic in marine systems represent a desirable technology, encouraging the search for novel microorganisms with these features. The shallow hydrothermal system off the Greek island Milos expels fluids of high temperature and salinity with extremely elevated arsenic concentrations, identifying this environment as an ideal natural laboratory to search for endemic microbes with bioremediation potential. Recent data indicate lipids as primary focus for microbial adaptation to these extreme conditions.

Objectives

This study focusses on a novel halophilic isolate from the Milos hydrothermal system, its membrane lipid adaptations under arsenic stress and its potential for bioremediation applications.

Methods

The bacterial isolate was physiologically characterized and identified by 16S rDNA gene sequencing. Arsenic precipitation was evaluated by scattering electron microcopy couple to energy dispersive X-ray spectroscopy for shape, size and elemental composition determination. Microbial lipids were extracted in the stationary growth phase and response to arsenic stress (20 mM) and increasing salt concentrations (2.5 to 20%) were investigated by high performance liquid chromatography coupled to mass spectroscopy.

Conclusions

A novel moderately halophilic bacterium (optimal growth at 5% NaCl), exhibits an unusual high As(V) tolerance (tested up to 20 mM) associated with generation of Mg-As-O rich particles, needle shape and size near to 100 nm length. pH dependent co-precipitation of Mg and As is suggested as bioremediation mechanism. Composition of intact polar lipids showed unique profiles, indicating saturation of methyl-branched fatty acids as adaptation to salt stress and a significant decrease of respiratory quinones under increasing arsenic stress.

FEMS7-2699
Environmental Microbiology/Microbial Ecology /Microbial Communities

UNRAVELING THE MECHANISMS OF HOST-INTERACTION IN THE MACROALGAL PATHOGEN NAUTELLA SP. R11

S. Egan[1], J. Hudson[1], M. Gardiner[1], N. Deshpande[2]
[1]University of New South Wales, School of Biological- Earth and Environmental Science, Sydney, Australia
[2]University of New South Wales, School of Biotechnology and Biomolecular Sciences, Sydney, Australia

Backgrounds

Macroalgae are essential for the functioning of temperate marine ecosystems, but their survival is under threat as a result of anthropogenic pressures and disease. *Nautella* R11 is recognised as one etiological agent responsible for the occurrence of bleaching disease in the red alga, *Delisea pulchra*. Previous work has suggested that bacterial quorum sensing (QS) is involved in regulating *Nautella* R11 virulence, yet there is a lack of knowledge of specific virulence mechanisms involved in this host pathogen interaction.

Objectives

To determine the role of the LuxR- type QS regulator RaiR in the regulation of *Nautella* R11 virulence and to identify the transcriptional response of this pathogen to the presence of its macroalgal host.

Methods

A *Nautella* R11 *raiR* gene deletion strain was created and assessed for its ability to cause bleaching disease in *D. pulchra* relative to the wildtype strain. We used a RNA-sequencing approach to assess the transcriptional response of both widtype and mutant strains in the presence and absence of the host.

Conclusions

Mutations in *raiR*, render *N. italica* R11 avirulent, suggesting this gene is important for regulating the expression of virulence phenotypes. We found that genes related to oxidative stress resistance, carbohydrate uptake and central metabolism are all upregulated in *N. italica* R11 in response to the macroalgal host and maybe play an important role towards the virulence of this opportunistic pathogen. Furthermore RaiR appears to regulate a subset of these genes, including those involved in phenylacetate degradation, galactose metabolism, oxidative stress response and also controls the expression of multiple phage proteins. Interestingly a large proportion of RaiR controlled genes were annotated as hypothetical or predicted function only, suggesting the presence of yet uncharacterised virulence mechanisms in this marine bacterium. The outcome of this research contributes to our greater understanding of the pathogenic traits mitigating microbial diseases in marine ecosystems.

FEMS7-0943
Environmental Microbiology/Microbial Ecology /Microbial Communities

OLIGOTYPING TO ANALYZE LAB SUCCESSION OF RDNA VARIANTS THROUGHOUT ATOLE AGRIO FERMENTATION

P. Elizaquivel[1], A. Perez-Cataluña[2], P. Carrasco[2], J. Espinosa[3], D. Reyes[4], C. Wacher[5], R. Aznar[6]
[1]Colección Española de Cultivos Tipo - Universidad de Valéncia, Microbiology and ecology, Paterna Valencia, Spain
[2]Universidad de València, Microbiology and ecology, Burjassot, Spain
[3]Universidad Juárez Autónoma de Tabasco, Unidad Sierra. Villahermosa, Tabasco, Mexico
[4]Universidad Autónoma Metropolitana Unidad Cuajimalpa, Departamento de Procesos y Tecnología, México- D.F., Mexico
[5]Universidad Nacional Autónoma de México, Departamento de Alimentos y Biotecnología- Facultad de Química, México D.F., Mexico
[6]Colección Española de Cultivos Tipo - Universidad de Valéncia, Microbiology and ecology, Burjassot- Valencia, Spain

Backgrounds

Atole agrio is a Mexican non-alcoholic beverage derived from fermented maize. Lactic acid bacteria (LAB) are commonly found as a major population in this product. Comprehension of microbial ecology of *Atole* and, in general, LAB fermented products, would help to the implementation of new food manufacturing processes and possible biotechnological applications of LAB strains.

Objectives

To track the intraspecific population dynamics of predominant genera, *Weissella* and *Lactobacillus,* throughout *Atole* fermentation using oligotyping as an annotation independent method.

Methods

Partial 16S rRNA gene sequencing (V3–V5) was obtained from DNA directly extracted from ten *Atole* samples, corresponding to 0h, 4h, 6h, 12h and 24h fermentation from each liquid and solid batches. A total of 63,952 reads with an average length of 528 bp were obtained and analyzed (41,613 for liquid fermentation and 22,339 for solid fermentation). Oligotyping was performed using the open-source software pipeline available at http://merenlab.org/projects/oligotyping/.

Conclusions

For *Lactobacillus,* two oligotypes (C and T) were found. Oligotype C predominated at the beginning of the fermentation, but decreased after 12h and imposed at the end of fermentation. On the contrary, oligotype T increased to the maximum at 12h and decreased throughout the end.

Regarding *Weissella,* two oligotypes (C and G) were found. Oligotype (C) predominated at the beginning of the process but dropped at 6h and raised again outnumbering oligotype G at the end of the fermentation. In both species, the predominant oligotype detected at the initial step became predominant at the end of the process; despite they evolved differently during the fermentation.

FEMS7-0514
Environmental Microbiology/Microbial Ecology /Microbial Communities

CO-CULTURE GROWTH BETWEEN COMMENSAL ESCHERICHIA COLI AND SEROTYPE O157:H7 ISOLATES REVEALS A COMPETITIVE EDGE FOR NON-PATHOGENIC STRAINS.

T. Ells[1], D. Albert[2], M. Ritchie[1], D. Mader[1], H. Eberl[3], G. Bezanson[1], M. Kalmokoff[1]
[1]Agriculture and Agri-Food Canada, Food Safety, Kentville- NS, Canada
[2]Acadia University, Department of Biology, Wolfville- NS, Canada
[3]University of Guelph, Department of Mathematics and Statistics, Guelph- ON, Canada

Backgrounds

Escherichia coli are common constituents of the mammalian enteric community. Serotype O157:H7 differs from commensal *E. coli* by its ability to produce shiga-like toxins and cause serious illnesses in humans. Although ruminants represent the primary reservoir for serotype O157:H7 it does not incite disease in these animals. Serotype O157:H7 can be isolated from beef and dairy cattle, but not all animals carry the pathogen and questions remain regarding its carriage.

Objectives

Examine the growth kinetics of a diverse collection of commensal *E. coli* and serotype O157:H7 isolates to elucidate potential interactions during competition for a similar niche.

Methods

E. coli isolates (~1000) were obtained from fecal samples from an experimental beef herd in Nappan, Nova Scotia. Isolates were initially screened by rep-PCR and then by PFGE. Growth kinetics for all representative pulsotypes as well as a 51 strain panel of O157:H7 isolates were assessed using impedance technology (RABIT). Competitive growth dynamics were evaluated between selected co-culture pairings of commensal versus serotype O157:H7 strains by end point enumerations on BCIG.

Conclusions

Fifty nine unique pulsotypes were identified among the commensal *E. coli* isolates. Monoculture impedance growth curves revealed these isolates generally had significantly higher growth rates (and a narrower range) than the panel of O157:H7 strains. Competition studies between selected commensals paired with each of 32 different O157:H7 isolates resulted in end point strain ratios favoring commensal strains by varying degrees. Although data suggest an advantage for commensal strains further studies are required to elucidate the impact of substrate variation on competition outcomes.

FEMS7-2654
Environmental Microbiology/Microbial Ecology /Microbial Communities

COMPARISON METHODS FOR THE EFFICACY TESTING OF OIL INDUSTRIAL BIOCIDES (LABORATORY EVALUATION)

I. Elmisurati[1], *H. Althalb*[2]
[1]*Libyan Petroleum Institute, Industrial &Production Chemistry, Tripoli, Libya*
[2]*Libyan Petroleum Institute, Industrial & Production Chemistryt, Tripoli, Libya*

Backgrounds

The disinfection efficacy of chemical biocides and the subsequent assessment of the metabolic state of the microorganisms can be evaluated by a variety of methodological approaches.

Objectives

The action of the bactericides was tested against planktonic sulphate reducing bacteria isolated from oil field. The ability of the planktonic SRBs to recover from the biocidal application was quantified by determining SRA (SO_4^{-2}) as the terminal electron acceptor in an aerobic respiration.

Methods

Most probable number (MPN) in terms of Time-kill test for culturable bacteria, fluorochrome-based assay by (DAPI) or (AO) with aldehyde and non aldehyde based biocides has been used to measure total bacterial count.

Biocide is incompatible with oxygen scavenger; however biocidal activity is lost upon addition of oxygen scavenger.

GM bacteria such as *Escherichia coli*HB101 pUCD607 and naturally occurring bacteria such as *Vibrio fischeri* has been used as a biosensors to detect toxicity of biocides.

Conclusions

Considerable variation is seen in the activity of the tested biocides with these bioluminescent strains, this assay may be useful not only in determining the concentration of biocides in oil-water system, but also in determining optimal biocide concentrations on a real-time basis.

FEMS7-1507
Environmental Microbiology/Microbial Ecology /Microbial Communities

A NOVEL STRAIN OF MODERATELY HALOPHILIC BACTERIA BELONGING TO GARICOLA GENERA ISOLATED FROM HISTORICAL FRESCOES

M. Enache[1], R. Cojoc[1], S. Neagu[1], I. Gomoiu[2]
[1]Institute of Biology Bucharest - Romanian Academy, Microbiology, Bucharest, Romania
[2]National Arts University Bucharest, Arts Conservation, Bucharest, Romania

Backgrounds

Moderately halophilic bacteria are spread in various media containing sodium chloride and other salts from negligible quantity until saturation. The genus *Garicola* was proposed in 2015 by Lo et al., based on the isolation of a novel strain from traditional Korean fermented shrimp. From the mural painting of Hurezi Monastery in Romania, building between years 1690 – 1693, were isolated several strains of bacteria able to grow on media with salt concentration until to 4M.

Objectives

This work deals with characterization of a moderately halophilic bacteria strain in order to propose it as a new species. On the other hand the methodologies for conservation of the mural paint in order to avoid the future development of microbial strains are considered.

Methods

The novel strain is characterized by proposed minimal standard for new taxa of moderately halophilic bacteria. The various biochemical tests were performed, G+C content, DNA-DNA hybridization test, 16S rDNA sequence, membrane lipid and protein profiles and other tests.

Conclusions

Preliminary results based on 16S rDNA investigations, BLAST analysis and some biochemical tests showed that novel strain is close related to *Garicola* genera. Considering all data obtained the strain is proposed as a novel species of this genus.

Acknowledgements:
This work was supported by Program Partnerships in Priority Domains – PN II developed with the support of MEN-UEFISCDI, Project no. 273/2014 and 323/2014 and project no. RO1567-IBB05/2016 from the Institute of Biology Bucharest of Romanian Academy.

FEMS7-0867
Environmental Microbiology/Microbial Ecology /Microbial Communities

FRUCTOPHILIC LACTIC ACID BACTERIA: TAXONOMY, BIOCHEMICAL AND GENOMIC CHARACTERISTICS AND POSSIBLE APPLICATION

A. Endo[1], S. Maeno[1], J. Nakagawa[1], S. Salminen[2], L. Dicks[3]
[1]Tokyo University of Agriculture, Food and Cosmetic Science, Hokkaido, Japan
[2]University of Turku, Functional Foods Forum, Turku, Finland
[3]Stellenbosch University, Microbiology, Stellenbosch, South Africa

Backgrounds

Fructophilic lactic acid bacteria (FLAB) are a special group of lactic acid bacteria (LAB) found only in fructose-rich niches, which have been established recently. Their specific characteristics had not been well documented.

Objectives

Our research is to study their unique characteristics and adaptation to their habitat.

Methods

Biochemical and genomic characteristics of FLAB were studied. FLAB prefer fructose but not glucose as a growth substrate. External electron acceptors, e.g. pyruvate, support the growth of the organisms on glucose. Moreover, the presence of O_2 strongly enhances their growth, although they do not possess respiratory chain. All five species of the genus *Fructobacillus* and *Lactobacillus kunkeei* are classified in this interesting bacterial group. The genomic characterization of *Fructobacillus* spp. as compared to their phylogenetic relatives, *Leuconostoc* spp., revealed their unique genomic evolution, e.g. less CDS and poor systems in carbohydrate transport and metabolism. The *adhE* gene encoding bifunctional alcohol/acetaldehyde dehydrogenase, which is essential for regeneration of NAD in glucose metabolism of heterolactic metabolism, is missing in all *Fructobacillus* spp. They are the first organisms which lack *adhE* gene in the heterofermentative LAB group. FLAB are found in flowers and fruits and also major components of gut microbiota in insects consuming fructose rich diets, i.e. honeybees. Our preliminary study revealed that certain FLAB strains specifically inhibit the growth of European foulbrood pathogen, *Melissococcus plutonius*.

Conclusions

FLAB possess quite unique biochemical and genomic characteristics in LAB group, and this would be due to a regressive evolution during adaptation to their fructose-rich habitats.

FEMS7-1424
Environmental Microbiology/Microbial Ecology /Microbial Communities

ENTEROBACTERIACEAE AND ANTIBIOTIC RESISTANCES ON LEAFY GREENS

A. Erlacher[1], *M. Grube*[2], *G. Berg*[1]
[1]*Graz University of Technology, Institute of Environmental Biotechnology, Graz, Austria*
[2]*University of Graz, Institute of Plant Sciences, Graz, Austria*

Backgrounds

Plants live in close interactions with microbial communities that are essential for the performance and survival of the host. However, these diverse microbiomes of vegetables can also act as reservoirs for antibiotic resistances, opportunistic and emerging pathogens. Increased consumption, larger scale production and efficient logistics of vegetables are at risk for contamination with pathogens causing human disease.

Objectives

Therefore, a comprehensive understanding of microbial structure on food items is urgently needed.

Methods

Using next generation sequencing techniques including, sequencing of 16S rRNA amplicons, whole metagenome shotgun sequencing, and complementary FISH/CLSM analyses, we were able to shed light on the structural and functional lifestyle of enterobacteria on fresh produce.

Conclusions

We found general preference of *Enterobacteriaceae* to colonize vegetables as abundant, highly diverse and indigenous key taxa, which are highly sensitive responders to biotic stress. CARD analysis complemented with disc diffusion antibiotic sensitivity testing revealed an enrichment of antibiotic resistances on the phyllosphere. Here, efflux pump conferring antibiotic resistances were predominant, followed by fluoroquinolone and chloramphenicol resistance genes. Further, an interesting colonization behavior was observed, as enterobacteria selectively intrude foliage through bruises, vascular bundles or stomata. Microbial network analyses of lettuce root samples revealed loose microbiome structures indicating susceptibility to perturbation and potential pathogen infestation. Our studies provided important insights to understand the nature of potential human pathogens and occurrence of antibiotic resistances in plant-associated ecosystems.

FEMS7-0928
Environmental Microbiology/Microbial Ecology /Microbial Communities

LISTERIA MONOCYTOGENES MAINTAINS ITS VIRULENCE POTENTIAL WHEN IT IS TRANSMITTED ALONG THE MICROBIAL FOOD CHAIN

S. Ermolaeva[1], V. Pushkareva[1], J. Podlipaeva[2], A. Gudkov[2]
[1]Gamaleya Research Center of Epidemiology and Microbiology,
Department of Infections with Natural Foci, Moscow, Russia
[2]Institute of Cytology, Laboratory of Cytology of Unicellular Microorgaisms, St.Petersburg, Russia

Backgrounds

Unicellular predators including *Amoeba* spp and ciliates feed on bacteria and smaller protozoa and might control pathogenic bacteria in natural ecosystems. Some pathogens seem to develop mechanisms that protect them from protozoa. The same mechanisms might be involved in virulence for mammals and humans. The thiol-dependent haemolysin, Listeriolysin O (LLO) is a key virulence factor of Gram-positive pathogenic bacterium *Listeria monocytogenes*. LLO causes mortality and encystment of *Tetrahymena pyriformis* feeding on LLO-producing bacteria (Puhkareva & Ermolaeva 2010).

Objectives

To establish a *L. monocytogenes* ability to maintain its virulence potential when it is transmitted along the microbial food chain and the role of LLO.

Methods

Wild type and LLO-lacking *L. monocytogenes* and wild type and LLO-producing *L. innocua* strains were used to feed *T. pyriformis* strain GL. Then washed and gentamycin treated bacterium-containing *T. pyriformis* were used to feed *Amoeba proteus* strain LB1503/4/.

Conclusions

A. proteus when fed on *T.pyriformis*, which in turn was fed on LLO-lacking bacteria, corresponded to normal physiological parameters (motility, size, the number of pseudopodia) up to 72 h. Feeding on T.*pyriformis* fed on LLO-producing bacteria caused loss of pseudopodia in 24 h and amoeba destruction in 72 h with releasing alive bacteria. Therefore, (i) *L.monocytogenes* maintained its virulence potential when it was transmitted along the microbial food chain, and (ii) the fate of *A. proteus* fed on bacterium-carrying ciliates was determined by LLO production. Obtained results proved the role of protozoa as a Trojan horse and demonstrated how a pathogen can move along a food chain.

FEMS7-0568
Environmental Microbiology/Microbial Ecology /Microbial Communities

IN SITU STUDY OF IBERIAN PYRITE BELT HARD ROCK BIOFILMS BY FLUORESCENCE MICROSCOPY TECHNIQUES

C. Escudero[1], *M. Oggerin de Orube*[1], *R. Amils Pibernat*[1]
[1]*CBMSO-UAM, Virología y Microbiología, Madrid, Spain*

Backgrounds

Natural microbial communities are characterized by the co-existence of microorganisms in self-produced matrix named biofilms. This matrix is compounded by extracellular polymeric substances (EPS) that create an ideal microenvironment in which microorganisms can survive even in adverse conditions.

Subsurface is an extreme environment where there is no sunlight or oxygen and organic matter is scarce or no bioavailable. Life is limited to low energy anaerobic metabolisms and the principal energy source depends on the geochemistry of the system. For this reason, it is believed that microorganisms present a very low metabolic rate or remain in a latent state and, consequently, biofilms are not produced due to the energetic cost required.**Objectives**

IPBSL is a drilling project designed to characterize the Iberian Pyrite Belt (IPB) subsurface ecosystem, which is responsible of the extreme acidic conditions of Río Tinto basin. The aim of this study was to analyze, *in situ*, the biodiversity and the presence of biofilms in subsurface hard rock matrix.

Methods

Fluorescence *in situ* Hybridization (FISH), CAtalyzed Reporter Deposition-FISH (CARD-FISH) and Fluorescence Lectin Binding Assay (FLBA) were applied in rock samples of different depths.

Conclusions

CARD-FISH, which allows the identification of microorganisms due to the use of specific probes, has shown the existence of a high microbial biodiversity in the IPB subsurface. Besides, the combination of FISH techniques and FLBA reveal that most of these microorganisms are part of mixed biofilms. These results suggest that production of biofilms can be a good structural mechanism for microbial activity and survival in deep subsurface environments.

FEMS7-1590
Environmental Microbiology/Microbial Ecology /Microbial Communities

EFFECT OF AIR POLLUTION ON BACTERIAL COMMUNITIES ASSOCIATED WITH PLATANUS HISPANICA (LONDON PLANE) LEAVES

J. Espenshade[1], L. Mattens[2], S. Thijs[1], N. Weyens[1], J. Vangronsveld[1]
[1]Hasselt University, Centre for Environmental Sciences, Hasselt, Belgium
[2]University of Leuven, Department of Pharmaceutical and Pharmacological Sciences, Leuven, Belgium

Backgrounds

Many plant-associated bacteria have a positive effect on the host plant's health and growth by producing useful biomolecules. It is also hypothesized that when these bacterial communities are constantly exposed to air pollution, they will favour strains that have a higher tolerance against and possibly degrade some polluting compounds. In this study, we searched for plant growth promoting leaf-associated bacteria with laccase-like multicopper oxidase enzymes (LMCO), which reportedly have the potential to degrade polycyclic aromatic hydrocarbons (PAHs) from diesel exhaust.

Objectives

The aim of this study was to isolate endophytic bacteria from *Platanus* leaves and characterise them for traits related to degradation of air pollution and promotion of plant health.

Methods

Platanus leaves were collected inside and outside of the city of Hasselt, Belgium. Bacteria were isolated from pulverised leaf material exposed to diesel fumes. Isolated bacteria were genotyped and screened for ACC-deaminase and auxin production, phosphate solubilisation, PAH tolerance, and laccase-like enzyme activity.

Conclusions

Initial results indicate that the *Platanus* tree outside the city centre hosted a larger bacterial community than the trees close to roadways. However, trees that were more directly exposed to air pollution appear to not only host bacteria with a greater tolerance for diesel fumes but also a greater proportion of bacteria with plant growth promoting traits and potential LMCO enzymes.

FEMS7-0927
Environmental Microbiology/Microbial Ecology /Microbial Communities

ISOLATION AND CHARACTERIZATION OF SHEWANELLA PUTREFACIENS GROUP FROM DISEASED WILD EELS AND FRESHWATER LAKE WATER

C. Esteve[1], *E. Alcaide*[1]
[1]*Universitat de València, Microbiología y Ecología, Burjassot, Spain*

Backgrounds

Shewanella putrefaciens group are commonly found in brackish/marine water and sediments where they are important in the process of organic matter turnover under hypoxic conditions. *Shewanellae* are also present in wild marine fish intestinal microbiota where they may have an antagonistic effect on bacterial pathogens. At present, the role of *S. putrefaciens* group as fish pathogen is only based on infections of freshwater farmed fish that are suffering from non-determined stress conditions

Objectives

There are two objectives: 1) to assess the role of *Shewanellae* as pathogens of the European eel fishery at present, 2) to determine the environmental conditions at the L´Albufera Lake that favor an outbreak of such disease.

Methods

Physico-chemical parameters of water (i.e. O_2 dissolved; temperature: pH; conductivity; salinity) were measured *in situ*. Isolation and counting of *Shewanellae* from fish and water samples were performed by culturing on general (TSA-1) and selective (SH30VA) media. Bacterial isolates were assigned to *Shewanella* species by using conventional tests, API 20E and 20NE strips (Biomerieux), and 16S rRNA sequencing. Statistical analyses (correlation and regression) were used. Virulence for eel of *Shewanellae* isolates was assessed by both intraperitoneal (IP) injection, and bath immersion (BI) challenges.

Conclusions

Shewanellosis at the European eel fishery is promoted by hypoxic conditions in the aquatic system. First, the rise of *Shewanellae* in lake freshwater is correlated with hypoxia as general rule. Second, this fish infection can be transmitted through the water.

FEMS7-0696
Environmental Microbiology/Microbial Ecology /Microbial Communities

INCREASING BIOREMEDIATION CAPACITY IN HYDROCARBON POLLUTED SOIL MATRIX

C.O. Esuola[1,2,3], J. Trögl[2], M. Schlömann[1], O.O. Babalola[3], D. Tischler[1]
[1]Technical University Bergakademie Freiberg- Institute of Biosciences, Environmental Microbiology, Freiberg, Germany
[2]Jan Evangelista Purkyně University, Faculty of Environment, Ústí nad Labem, Czech Republic
[3]North-West University- Mafikeng Campus, Department of Biological Sciences, Mmabatho, South Africa

Backgrounds

Toxic, heavy metals and hydrocarbons are still employed in many industries which anthropogenically leak into the environment. More research efforts are still needed to elucidate the role of particular bacteria for effective bioremediation of hydrocarbon and metal polluted soils.

Objectives

To investigate and determine the rate at which both *Gordonia* sp. and *Rhodococcus* sp. are effective for biodegradation of high concentrations of petroleum hydrocarbons in contaminated soil under the influence of metal-stress.

Methods

Contaminated soil (500 kg) were collected from an rafirenry lagoon and prepared for the pot experiment. A total of twelve variants, each in four replicates, was realized. 500g of soil was weighed into each pot (48 pots). The soil without addition of bacteria served as the control. Aliquot sampling were collected from each pot from 0-49 days. Samples were analysed for dehydrogenase activity, respiration activity, dry weight, aliphatic hydrocarbon (C_{10}-C_{40}) degradation and metal mobility by sequence extraction and ICP-MS. Data were evaluated using confidence intervals (alpha=0.05) which allows pair comparisons of significance. Bioaugmentation, iron-stress and biocarrier (activated charcoal) enhanced dehydrogenase and respiration activity. The tested soil has a high concentration of Ca, Fe, Al, Mg. Other Trace elements, including As are also present at low concentrations. After 49 days, variant GR1; *Gordonia* sp. cultured in iron-contained bacteria salt media (BSM) added to soil, showed the highest degradation of the aliphatic hydrocarbons from ~80,000 mg/kg dry weight of soil to ~54,000 mg/kg.

Conclusions

Gordonia sp. and *Rhodococcus* sp. offer a biological degradation of industrially contaminated soil even under metal stress.

FEMS7-1633
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROBIAL FUNCTIONAL DIVERSITY AND ENZYMATIC ACTIVITIES IN A POST-COAL MINING RECLAMATION LAND CHRONOSEQUENCE

O. Ezeokoli[1], R. Adeleke[2], C. Bezuidenhout[1], M. Maboeta[1]
[1]North-West University, Environmental Sciences and Management, Potchefstroom, South Africa
[2]Agricultural Research Council-Institute for Soil- Climate and Water,
Microbiology and Environmental Microbiology, Pretoria, South Africa

Backgrounds

The restoration of soil ecosystem functioning in disturbed lands is crucial for a meaningful post-disturbance land use sustainability. Microbial community diversity and activity in soils are robust indicators for assessing soil ecosystem health and functional plasticity. Previous soil health assessments of reclamation lands have been based on aboveground indicators which are rather conservative.

Objectives

In this study, soil microbial functional diversity and enzyme activities of a post-coal mining reclamation land chronosequence (0- 20 years) located in the coal-rich Highveld of Mpumalanga, South Africa were investigated in summer.

Methods

Functional diversity was determined using utilization profiles of 31 carbon substrates on Biolog EcoPlates. Activities of β-glucosidase, alkaline- and acid- phosphatases and urease in bulk soil were assayed.

Conclusions

Mean Shannon-Weiner index (H´) and evenness (J) were lower in the acidic chronosequence soils compared to undisturbed lands. Within chronosequence, differences in H´ and J were not significant ($P > 0.05$), with H´ inversely related to reclamation years and J increased between the first two years of reclamation. In general, enzymatic activities significantly differed ($P < 0.05$) between reclamation chronosequence and undisturbed lands. β-glucosidase activity (p-nitrophenol µg/g/h) ranged from 66.8 to 524.20, and from 409.79 to 693.83 in reclaimed and undisturbed soils, respectively. Urease activity increased with reclamation years and was lower in reclaimed lands. Similarly, alkaline phosphatase and acid phosphatase activities reflected gradients indicative of phosphorus availability and vegetation growth. Changes in functional diversity and enzymatic activities within the chronosequence suggests development of a functionally redundant microbial community driving nutrient cycling within the reclamation soil ecosystem.

FEMS7-3073
Environmental Microbiology/Microbial Ecology /Microbial Communities

ENZYME ACTIVITIES AND MICROBIAL DIVERSITY IN SOUTH AFRICAN COAL MINE SOIL STOCKPILES

S.K. Mashigo[1,2], R. Adeleke[1,2], E. Obinna[1,2], C.C. Bezuidenhout[2]
[1]Agricultural Reasearch Council, Institute for Soil- Climate and Water, Pretoria, South Africa
[2]Environmental Sciences and Management, North-West University- Potchefstroom campus, North-West, South Africa

Backgrounds

The quality of stockpiled soils are important for a meaningful post-mining land reclamation. The topsoil layer is required to be removed and stockpiled separately from other overburden material stockpiles. Soil stockpiles are often stored for longer period of time and the removal and replacement of the soil layers with heavy equipment's are factors that affect soil quality from an agricultural- use standpoint.

Objectives

The study was undertaken in order to determine the possible impact of stockpiling on the functional and diversity of microbiota (bacteria and fungi) in stockpiled soils of coal mines.

Methods

Soil samples were randomly collected from three opencast coalmines stockpile soils at a depth of 0-20 cm ("topsoil") and >20 cm ("subsoil"). Unmined soil served as a control. The physical and chemical characteristics of the soils, including soil texture, bulk density, total organic carbon, and pH, were determined. Microbial community functions were determined using enzyme assays (β-glucosidase and urease). Microbial diversity was determined using PCR-denaturing gradient gel electrophoresis (DGGE).

Conclusions

The physical and chemical properties of unmined soils significantly ($P<0.05$) varied from that of stockpile soils. The enzymatic activities (β-glucosidase and urease) were higher in stockpiled soils than unmined soils. Densitometry analysis of DGGE snapshots revealed higher microbial diversity in unmined (control) soils. The results suggest that the stockpiling process negatively affects microbial diversity. The higher enzymatic activities in stockpiled soils suggest nutrient deficiency. Overall, these findings demonstrate that soil stockpiling process has potential negative effects on soil quality.

FEMS7-0586
Environmental Microbiology/Microbial Ecology /Microbial Communities

FEEDING THE BEAST TO KEEP THE BEAUTY! GENOME EVOLUTIONARY DYNAMICS OF ENDOSYMBIOTIC BACTERIA OF INSECTS

M. Fares[1], B. Sabater-Muñoz[1], C. Toft[2], D. Alvarez-Ponce[3]
[1]Instituto de Biología Molecular y Celular de Plantas, Abiotic stress, Valencia, Spain
[2]Universidad de Valencia, Genetics, Valencia, Spain
[3]University of Nevada- Reno, Biology, Reno, USA

Backgrounds

The interactions between biological entities and their role in evolution have enthralled scientists for decades, but the causes and consequences of such interactions remain poorly characterized. An open question is how does the selection-drift balance determine the fates of biological interactions.

Objectives

We searched for signatures of selection and drift in genomes of five endosymbiotic bacterial groups to determine the role of chance and necessity in the evolution of endosymbiotic genomes.

Methods

We identified signatures of selection in endosymbiotic genes using maximum-likelihood approaches. The effect of drift was determined through experimental evolution of *Escherichia coli* populations. The evolution of regulation was described through RNA sequencing.

Conclusions

Many genes in endosymbiotic bacteria exhibited stronger selective constraints than their orthologs in free-living bacterial relatives. Remarkably, most of these genes had no role in the host-symbiont interactions, but were involved in either buffering the deleterious consequences of drift or other host-unrelated functions, suggesting that they have either acquired new roles or their roles became more central in endosymbiotic bacteria. Experimental evolution of *Escherichia coli* under strong genetic drift revealed remarkable similarities in the mutational spectrum and genome reduction patterns to endosymbiotic bacteria of insects. Experimentally evolved lines showed a generalized de-regulation of the genome that affected genes encoding proteins involved in mutational buffering, regulation and amino acid biosynthesis, patterns identical to those found in endosymbiotic bacteria. Our results indicate that drift has shaped endosymbiosic associations through a change in the functional landscape of bacterial genes, and that the host had a small role in this shift.

FEMS7-0590
Environmental Microbiology/Microbial Ecology /Microbial Communities

CELLULAR RESPONSES AND EVOLVED ADAPTATIONS OF SACCHAROMYCES CEREVISIAE TO GLYCEROL-INDUCED STRESS

M. Fares[1], F. Mattenberger[1], B. Sabater-Muñoz[1], J. Hallsworth[2]
[1]Instituto de Biología Molecular y Celular de Plantas, Abiotic stress, Valencia, Spain
[2]Queen´s University of Belfast, School of Biological Sciences, Belfast, United Kingdom

Backgrounds

Glycerol synthesis is key to central metabolism and stress biology in *Saccharomyces cerevisiae*, yet the cellular adjustments needed to respond and adapt to glycerol stress are little understood. Glycerol stress can result from an increase of glycerol concentration in the medium due to the *S. cerevisiae* fermenting activity or other yeast metabolic activities.

Objectives

Here, we determined impacts of acute and chronic exposures to glycerol stress in *S. cerevisiae*.

Methods

We subjected experimentally evolved *S. cerevisiae* populations to acute and chronic exposures to glycerol. We determined the effects of glycerol on *S. cerevisiae* fitness and identified transcriptomic alterations involved in the response and adaptation to glycerol.

Conclusions

Acute glycerol-stress led to a 50% decline in growth rate and altered transcription of more than 40% of genes. The increased genetic diversity in *S. cerevisiae* population led to an increase in growth rate and altered transcriptome when such population was transferred to stressful media containing a high concentration of glycerol. Evolution of *S. cerevisiae* populations during a 10-day period in the glycerol-containing medium led to transcriptome changes and readjustments to improve control of glycerol flux across the membrane, regulation of cell cycle, and more robust stress response; and a remarkable increase of growth rate under glycerol stress. Most of the observed regulatory changes arose in duplicated genes. These findings elucidate the physiological mechanisms, which underlie glycerol-stress response, and longer-term adaptations, in *S. cerevisiae*; they also have implications for enigmatic aspects of the ecology of this otherwise well-characterized yeast.

FEMS7-1569
Environmental Microbiology/Microbial Ecology /Microbial Communities

MARINE PROSPECTING OF CELLULASE GENES: CAN THE RED SEA BE ANOTHER ENERGY RESERVOIR?

S. Fatani[1], Y. Saito[1], K. mineta[1], T. Gojobori[1]
[1]King Abdullah University of Science & Technology KAUST, BESE, Thuwal Jeddah, Saudi Arabia

Backgrounds

Cellulase is a key player in biorefinery, especially for the utilization of plant biomass. In the Middle East in particular, biofuel is considered to be one of the important alternative energies to the crude oil. Then, an interesting question is raised if cellulolytic microbes can be found in the Red Sea. Moreover, the Red Sea is a unique environment with a high temperature and salinity.

Objectives

The aim of the present study is to know whether the Red Sea can be a source of cellulase-active microorganisms.

Methods

Although we, first, isolated 700 different bacterial strains from seawater, no cellulase-producing strains were obtained. Then, we isolated 369 different isolates from plankton fraction of sweater using 0.63 µm mesh, successfully obtaining two cellulase-active strains. We also identified two more cellulase-active strains out of 300 bacterial isolates that were collected from seaweeds bodies. Small subunit rRNA gene sequences suggested that one of the strains is related to Ascomycota fungus whereas the other three isolates belong to Firmicutes bacteria. We showed that the hydrolysis observed in those four strains was actually caused by cellulases secreted.

Conclusions

In conclusion, the Red Sea is rich in cellulase-producing microorganisms, most of which are probably present on the surface of cellulose containing organisms such as algae. Therefore, the Red Sea can be potential reservoir of energy that is worthwhile being exploited in the coming years.

FEMS7-1853
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE INFLUENCE OF GEOGRAPHICAL ORIGIN AND SUBSTRATE PREFERENCES ON THE BIOCHEMICAL AND MOLECULAR GENETIC PLASTICITY OF BASIDIOMYCETE STRAINS - STECCHERINUM OCHRACEUM (PERS.) GRAY

T. Fedorova[1], O. Glazunova[1], K. Moiseenko[1], N. Shakhova[2], N. Psurtseva[2]
[1]Federal State Institution Federal Research Centre "Fundamentals of Biotechnology" of Russian Aca demy of Sciences, A.N. Bach Institute of Biochemistry, Moscow, Russia
[2]Komarov Botanical Institute of Russian Academy of Sciences, Laboratory of Fungal Biochemistry, St.Peterburg, Russia

Backgrounds

White-rot fungi as wood degraders are very important part of virtually all ecosystems. Studies of the physiological role and ecological adaptation mechanisms of white rot fungi attracted considerable interest of researchers in different areas. Basidiomycete *Steccherinum ochraceum* – is a species with great ecological amplitude. It occurs in different regions of Russia and in different climatic zones. *S. ochraceum* colonizes stumps, dead trunks and branches of various deciduous and seldom coniferous trees at different decay stages, but also could be found on living trees.

Objectives

The aim of our work was to examine genetic and biochemical diversity *S. ochraceum* strains from different geographic regions of Russia collected from different wood substrates.

Methods

The strains were obtained from Komarov Botanical Institute Basidiomycetes Culture Collection (LE-BIN). The phylogenetic diversity of *S. ochraceum* strains was examined by analyzing the nucleotide sequences of β-tub and *tef1α* gene and the nuclear ITS1-5.8SITS2 domain. Activity of enzymes was estimated for strains growing on liquid glucose–peptone medium and wood-supplemented growth medium.

Conclusions

Based on the concatenated dataset of all genes, the *S. ochraceum* strains were placed into three main clades correlating with lignocellulose-converting enzyme activity profiles of these strains: high oxidoreductase and low cellulose-converting activities; medium oxidoreductase and cellulose-converting activities; low oxidoreductase and high cellulose-converting activities. Thus, for the diverse *S. ochraceum* strains, there is a strong connection between the genotype and their lignocellulose-modifying activity profiles, but not with geographic origin of strains.
This work was supported by Grant of Russian Foundation For Basic Research 16-04-01184-a.

FEMS7-1926
Environmental Microbiology/Microbial Ecology /Microbial Communities

PRE-TERM BIRTH IS ASSOCIATED WITH DIFFERENT POPULATIONS OF VAGINAL MICROBIOTA COMPARED TO FULL-TERM BIRTH

C. Feehily[1,2], D.A. Crosby[3], F.M. McAuliffe[3], R.P. Ross[2], P.D. Cotter[1,2]
[1]Teagasc Food Research Centre, Food Bioscience, Cork, Ireland
[2]APC Microbiome Institute, University College Cork, Cork, Ireland
[3]Department of Obstetrics and Gynaecology- University College Dublin, National Maternity Hospital- Holles Street, Dublin, Ireland

Backgrounds

Bacterial vaginosis affects up to 30% of women worldwide and is associated with an increased risk of STI transmission, and adverse outcomes for pregnant women. There is a reported 2-fold increase of pre-term birth in women suffering BV during pregnancy. The condition is characterised by a shift in healthy populations of lactobacilli in the vagina towards a more mixed-species microbiota that mainly includes *Gardnerella vaginalis*, *Atopobium vaginae* and, *Prevotella* sp. amongst others.

Objectives

Our study aimed to examine the relationship between the vaginal microbiota and the onset of pre-term birth.

Methods

We recruited 30 pregnant women after 10 weeks gestation who were at risk of pre-term delivery and a further 21 pregnant women deemed not at risk. Using 16S amplicon sequencing on a MiSeq platform we compared the vaginal microbial composition between groups to determine any underlying microbial association with pre-term delivery.

Conclusions

Of the 9 women that subsequently delivered pre-term there were distinctly different patterns of lactobacilli populations compared with the microbiota found in full-term pregnancies. Specific groups of lactobacilli were only observed in the full-term cohort and absent in the pre-term cohort. There was no correlation observed between a BV state microbiota and gestational age at delivery.

FEMS7-2107
Environmental Microbiology/Microbial Ecology /Microbial Communities

RESPONSE OF A SALT MARSH BACTERIAL COMMUNITY TO SILVER AND COPPER NANOPARTICLES: IMPLICATIONS FOR PHYTOREMEDIATION PROCESSES

J. P. Fernandes[1,2], *A.P. Mucha*[1], *C. R. Gomes*[1,3], *C.M. R. Almeida*[1]
[1]*CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, EcoBioTec, Porto, Portugal*
[2]*ICBAS/UP - Institute for the Biomedical Sciences Abel Salazar / University of Porto, ICBAS, Porto, Portugal*
[3]*FCUP - Faculty of Sciences of the University of Porto, Department of Chemistry and Biochemistry, Porto, Portugal*

Backgrounds

Silver (AgNP) and copper oxide (CuONP) nanoparticles are among the most used and their presence in estuarine environment is expected. It is known that salt marsh plants can take up metals in a process known as phytoremediation and that this process can benefit from the microbial activity around plant roots. Nevertheless, depending on its levels and forms, metals can be toxic to microorganisms, fact that can eventually compromise their contribution to the phytoremediation processes.

Objectives

Present work aimed to investigate the response of the microbial community colonizing *Phragmites australis* rhizosphere to AgNPs and CuONP contamination, evaluating the implications for phytoremediation processes.

Methods

One week experiments were carried out with medium (elutriate solution with the respective sediment) doped with Ag or Cu, either in ionic or NP form. Microbial community was characterized in terms of bacterial diversity, richness and community structure through ARISA, and metal uptake was evaluated in plant tissues, elutriate solutions and sediments by atomic absorption spectroscopy.

Conclusions

Overall, the presence of Ag or Cu and the form in which the metal was added to the medium clearly affected the bacterial community structure, despite richness and diversity were only affected by the presence of Cu. Regarding metal uptake, no Ag accumulation was observed and significant Cu uptake was observed only in roots tissues when Cu was added to the medium in ionic form.

The presence of both metals in either forms affected the bacterial community structure, which may cause disturbances in ecosystems function and compromise phytoremediation processes.

Acknowledgments: PhD scholarship SFRH/BD/112154/2015 (FCT) and project NORTE-01-0145-FEDER-000035 (ERDF).

FEMS7-2151
Environmental Microbiology/Microbial Ecology /Microbial Communities

BIODEGRADATION OF PHARMACEUTICALS BY MICROORGANISMS ISOLATED FROM WASTEWATER TREATMENT PLANTS AND ESTUARINE SEDIMENTS

J. Fernandes[1,2], *P. Duarte*[2], *C.M. R. Almeida*[1], *M.F. Carvalho*[1], *A.P. Mucha*[1]
[1]*CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, EcoBioTec, Porto, Portugal*
[2]*ICBAS/UP - Institute for the Biomedical Sciences Abel Salazar / University of Porto, ICBAS, Porto, Portugal*

Backgrounds

In the last years, concerns about the detection of pharmaceuticals in the environment have grown, being one of the most relevant topics in environmental research. Bioremediation, the use of natural biological processes for ecosystems recovery, can lead to a partial or total elimination of contaminants from the environment.

Objectives

This work aimed to investigate the biodegradation of two pharmaceuticals, paroxetine and bezafibrate, by microorganisms isolated from an estuarine environment and an associated WWTP.

Methods

For that, isolated strains derived from microbial cultures previously enriched with the selected pharmaceuticals were reunited in 5
consortia: two consortia derived from paroxetine enrichment under static conditions, of which one was obtained from estuarine sediment and the other from activated sludge from WWTP, and three consortia derived from bezafibrate enrichment, two under static conditions, in which one was obtained from estuarine sediment and the other
from activated sludge, and the last obtained from activated sludge microcosms under agitation conditions.

Conclusions

Results showed that despite different acclimation times, high removal efficiency of pharmaceuticals (> 97%) was observed in three of the five studied consortia. Moreover, consortia derived
from estuarine sediment demonstrated to be more efficient in removing both studied pharmaceuticals. In the case of paroxetine, microorganisms present in consortia were able to promote fluoride anion release. Complementary analysis will allow the identification of the isolated strains present in the consortia responsible for bezafibrate and paroxetine degradation.

This work emphasizes the potential of microorganisms from estuarine environment and associated WWTP, for application in bioremediation techniques for pharmaceuticals removal.

Acknowledgments: PhD scholarship SFRH/BD/112154/2015 (FCT) and project NORTE-01-0145-FEDER-000035 (ERDF).

FEMS7-2427
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROBIAL DIVERSITY OF MINERO-MEDICINAL SULPHATED SPRING WATERS

C. Rodriguez[1], *M.C. De la Rosa Jorge*[2], *C. Pintado*[2]
[1]*University Complutense, Microbiology II, Madrid, Spain*
[2]*University Complutense Madrid, Microbiology II, Madrid, Spain*

Backgrounds

Mineral spring waters have been used since ancient times due to their health benefits. They present a complex biodiversity of microorganisms which depends on the water physicochemical characteristics. We have studied comparatively the microbial diversity and the chemical composition from 14 Spas and 22 Spanish natural springs classified as sulphated minero-medicinal waters, used in therapeutic treatments and balneotherapy.

This work has been done analyzing the data obtained for several years from multidisciplinary approaches with the participation of our research group and accomplished by the Mineromedicinal Waters Commission of the *Real Academia Nacional de Farmacia*.

Objectives

The main objectives have been: a) To compare the physicochemical characteristics of these sulphated waters in order to determine which are the parameters that regulate the biodiversity of these aquatic ecosystems. b) To study the microbial diversity, the sanitary quality indicator bacteria, pathogenic and those of ecological interest.

Methods

The sulphated springs studied come from different spas located throughout the Spanish geography (Albacete, Almería, Aragón, Castellón, Ciudad Real, Cuenca, Granada, Madrid, Navarra, Teruel, Valencia, Zaragoza). Culture, biochemical, optical and electron microscopy methods were used. Sensitivity and antibiotic resistance of opportunistic pathogenic strains was determined.

Conclusions

No pathogens nor faecal indicators were detected, in compliance with the Spanish regulations of drinking water. The populations were predominantly mesophilic and oligotrophic. Sulfate-reducing, proteolytic, amylolytic, cellulolytic, nitrifying and ammonifying bacteria, which contribute to water self-purification, were detected; also halophilic and iron bacteria and fungi. The large number of identified microbial species proves the great biodiversity of these waters.

FEMS7-0933
Environmental Microbiology/Microbial Ecology /Microbial Communities

THE ROLE OF CYCLIC DI-GMP DURING THE ESTABLISHMENT OF THE ENDOPHYTIC LIFESTYLE IN AZOARCUS SP. CIB

H. Fernández-Llamosas[1], *E. Díaz*[1], *M. Carmona*[1]
[1]*CIB-CSIC, Environmental Microbiology, Madrid, Spain*

Backgrounds

Azoarcus sp. CIB is a free-living facultative anaerobic β-Proteobacterium with a high metabolic versatility[1] and able to interact with plants as an endophyte[2]. Since the molecular determinants involved in plant-bacteria interactions are still poorly understood, there is much interest in studying the regulatory circuits controlling this process. Cyclic di-GMP (c-di-GMP) is a second messenger that regulates a large number of bacterial functions[3], but its role in the endophytic lifestyle of bacteria is still poorly known.

Objectives

Unraveling a new function of c-di-GMP in bacteria by measuring the effect of different c-di-GMP cellular levels in the endophytic lifestyle of *Azoarcus* sp. CIB

Methods

By cloning and expressing a heterologous c-di-GMP cyclase and phophodiesterase, we have generated recombinant CIB strains with higher or lower c-di-GMP levels, respectively, than the parental strain and that showed the expected phenotypes. Rice seedlings were in contact with CIB strains for ten days, and endophytes were recovered for CFU counting.

Conclusions

By artificially modulating the c-di-GMP levels in *Azoarcus* sp. CIB we have revealed that whereas an increase in c-di-GMP does not affect significantly the establishment of the bacteria inside rice roots, a decrease in c-di-GMP clearly decreased this interaction. This work provides new insights into the molecular basis of plant-bacteria interaction, and may contribute to engineer new strains with improved plant growth-promoting properties o for (endo)phytoremediation approaches

1. Martín-Moldes Z, *et al*. *Syst Appl Microbiol*. 2015;38(7):462-471.

2. Fernández-Llamosas H, *et al*. *PLoS One*. 2014;9(10):e110771.

3. Hengge R, *et al*. *Nat Rev Microbiol*. 2009;7(4):263-273.

FEMS7-3183
Environmental Microbiology/Microbial Ecology /Microbial Communities

GENETIC DIVERSITY OF NEW SSDNA VIRUSES FROM POLAR ENVIRONMENTS

C. Fernandez-Linares[1], *A. Rastrojo*[1], *A. Alcami*[1]
[1]*Centro de Biología Molecular Severo Ochoa, Virología, Madrid, Spain*

Backgrounds

Viruses are the most abundant biological entities on earth and play a major role as regulators of microbial communities. However, the current knowledge about them is still scarce, especially about their adaptation mechanisms and evolution. In this work we have studied the diversity and genetic variability of the viruses in several freshwater lakes in polar zones, isolated areas with very low human influence and with a low zoogenic input.

Objectives

The objective of this project is the study of the classification and variability of small circular ssDNA viruses found in several Antarctic and Arctic lakes.

Methods

Water samples were subjected to tangential flow filtration to remove cellular organisms and to concentrate viral fraction. Then viral DNA was extracted using phenol-chloroform method and was amplified using MDA before sequencing. Both 454 and Illumina technology were used, obtaining both single and paired reads. The subsequent viral metagenome assembly was carried out with the SPAdes program. Circular topology was identified using an in-house script based on the Minimus2 program. Genemark was used to predict putative genes, removing contigs without any potential gene. Viral genomes were then clustered based on sequence homology using the MCL program.

Conclusions

A large number of viruses with high variability have been found, although a specific relationship between the different viral families and their Shannon index was not observed. We have identified a large number of new viral families by classifying the contigs based on sequence homology.

FEMS7-1086
Environmental Microbiology/Microbial Ecology /Microbial Communities

STUDY OF ENZYMATIC ACTIVITIES AND BACTERIAL COMMUNITIES IN TWO FULL-SCALE MBR PLANTS TREATING WASTEWATERS FROM MUNICIPAL SOLID WASTES

J. Fernández-Navarro[1], L. Moreno-Mesonero[1], A. Zornoza[1], I. Amorós[1], J.L. Alonso[1], F. Muñagorri[2], C. Álvarez[2]
[1]Instituto Universitario de Ingeniería del Agua y Medioambiente, Universitat Politècnica de València, Valencia, Spain
[2]URBASER, URBASER, Madrid, Spain

Backgrounds

Municipal waste treatment generates wastewaters that have to be treated before their disposal. Membrane bioreactor systems (MBRs) are used for this type of complex wastewater treatment, because of their capacity of working at high concentrations levels of suspended solids and high cell retention times. However, this produces microbial matrixes with limited substrates which reduce the respiratory capability of bacterial communities.

Objectives

The study of enzymatic activities provide important information about the different metabolic processes occurring in the catabolism of proteins, carbohydrates, lipids, inorganic phosphorus assimilation and respiratory capacity of microorganisms.

Methods

Samples from activated sludge were collected monthly during fifteen months from two MBR systems. The enzymatic activities (phosphatases, lipases, glucosidases, proteases and dehydrogenases) were determined spectrophotometrically. The bacterial communities were identified by 16S rRNA gene amplicon sequencing. Multivariate analysis was used to reveal relathionships between the level of enzyme activities, volatile suspended solids (VSS), extracellular polymeric substances (EPS) and bacterial communities.

Conclusions

The activities of all the tested hydrolases were dynamic, showing diverse patterns of variation. Statistically significant relationships have been found between the population of microorganisms and the different enzymatic activities studied. Thus, the relative abundance of Firmicutes, Bacteroidetes and different classes of Proteobacteria has been associated with an increase in glucosidase, acid phosphatase and protease activities in the activated sludge of bioreactors. The simultaneous use of different enzyme activities is useful for the full evaluation of the biodegrading capacity of MBRs treating wastewaters from municipal solid wastes.

FEMS7-1674
Environmental Microbiology/Microbial Ecology /Microbial Communities

MICROBIAL HYDROLYTIC ENZYMATIC ACTIVITIES IN FOUR PILOT-SCALE SBR EXPERIMENTAL PLANTS UNDER ADDITION OF A METABOLIC UNCOUPLER

J. Fernández-Navarro[1], E. Ferrer-Polonio[2], I. Amorós[1], J.L. Alonso[1], J.A. Mendoza-Roca[2]
[1]Instituto Universitario de Ingeniería del Agua y Medioambiente, Universitat Politècnica de València, Valencia, Spain
[2]Instituto Universitario de Seguridad Industrial- Radiofísica y Medioambiental, Universitat Politècnica de València, Valencia, Spain

Backgrounds

Microbial growth of the active sludge is mainly generated by the organic matter present in the wastewater and the energy coming from the use of the dissolved oxygen as electron acceptor in the catabolism. This exponential growth becomes a problem in the wastewater treatment plants (WWTP) due to the expensive treatment of the biological sludge. For this reason, techniques for reducing the production of active sludge are studied, which do not imply a reduction in purification yields. The study of the microbial hydrolytic enzymatic activities (MHEA) offers on the one hand information about hydrolysis processes of the activated sludge and, on the other hand, valuable information useful to optimize conditions for a better process performance when a reduction of the sludge production is studied.

Objectives

To Monitor and evaluate the trend of enzymatic activities under different operating conditions when activated sludge is treated with TCS, a metabolic uncoupler agent used to reduce sludge production.

Methods

Samples from four SBR systems were collected weekly and MHEA were determined spectrophotometrically. MHEA were correlated with different F/M ratios, dissolved oxygen (DO) variations and TCS addition to the activated sludge.

Conclusions

TCS addition produces important variations of enzymatic activities, such as lipase, protease and acidic and alkaline phosphatase. Different concentrations of dissolved oxygen and F/M ratios have shown that both parameters are important for process control when sludge production is reduced. This indicates that the microorganisms are acclimatized to the presence of the metabolic uncoupler that produces improvements in organic matter removal performance.

FEMS7-0897
Environmental Microbiology/Microbial Ecology /Microbial Communities

DEVELOPMENT OF AN AGAR-BASED RNA-SEQ ASSAY FOR STUDYING THE INTERACTOME OF NASAL STAPHYLOCOCCI STRAINS

G. Hermansen[1], *L. Jelsbak*[1]
[1]*Technical University of Denmark, DTU Bioengineering, Kgs Lyngby, Denmark*

Backgrounds

Staphylococcus aureus is well-known as a human pathogen but also lives as a commensal in the human nose in approximately one fifth of the population. Carriage is usually asymptomatic but the nasal cavities can function as reservoirs for infection e.g. during surgical procedures. The human nose hosts a multi-species community and it has previously been reported that *Staphylococcus epidermidis* strains can modulate *S. aureus* phenotypes. Intriguingly the cohabitation has been shown to result in inhibition of nasal colonization and biofilm formation of *S. aureus*. Even though some *S. epidermidis* products have been implicated in this inhibitory effect, a comprehensive understanding of the interactions between nasal Staphylococci isolates is needed.

Objectives

The aim of this study was to establish a robust method to map the transcriptome profiles of nasal *S. aureus* and *S. epidermidis* focusing on the differences between mono- and co-culturing.

Methods

Staphylococci strains were isolated from nasal cavities of healthy Danish individuals. A pair of *S. aureus* and *S. epidermidis* strains isolated from the same nose were used for a novel agar-based RNA-seq method enabling investigation of their interactome. The strains were grown on agar surfaces as mono- and co-cultures, respectively, and RNA was harvested after 24 hours of incubation. RNA-seq was performed and enables mapping of the expression profiles of the two nasal Staphylococci isolates.

Conclusions

Our preliminary results indicate this novel RNA-seq method can be applied to study interactions between *S. aureus* and *S. epidermidis* isolated from nasal swaps of a healthy Danish adult.

FEMS7-2355
Environmental Microbiology/Microbial Ecology /Microbial Communities

ASSESSMENT OF COASTAL WATER QUALITY IN WOBURN BAY, GRENADA WEST INDIES, 12.0°N, 61.6°W

N. Bartholomew[1], A. Lin[2], B. Javier[2], K. Farmer-Diaz[1], M. Matthew[1], M. McCai[3], A. Kraus[3], S. Kotelnikova[1]
[1]St. George's University, Microbiology and Immunology, St. Georges, Grenada
[2]St. George's University, Medical Student Research InstituteMSRI, St. Georges, Grenada
[3]St. George's University, Medical Research Student, St. George's Grenada

Backgrounds

This was a novel study that was performed to establish a water quality baseline in Grenada by tracking sources of pollution as a means to maintain environmental health. Pollution management influences Grenada's overall economic sustainability, which is dependent on the environment. Potential eutrophication of water, due to anthropogenic activities, may lead to the rise of opportunistic pathogens and possibly neurotoxic effects on marine and human populations.

Objectives

To assess the coastal water quality at Woburn Bay and to identify the sources of pollution in three marine and two river sites.

Methods

Throughout eighteen weeks, that spanned the wet and dry seasons, twenty biochemical parameters, as well as sample, temporal and spatial variations were documented. Points of pollution were identified based on site cross-correlation, t-test and temporal variation analysis

Conclusions

The documented coliform MPN was above the EPA limits and positively correlated with rainfall data. BOD_5 levels were over 50 mg/l at river sites. Possible sources of non-point fecal pollution were the watershed runoff, septic tanks and recreational yachts which are permanently parked in the Bay. A significant relationship between rainfall and ammonium discharge was documented. An increased level of organic matter, phosphate, ammonium, sulfides, copper, and iron in the marine waters which further enhanced microbial oxidation was as a result of the mangrove filtering capacity failure. As a result, increased heterotrophic bacterial MPN counts, turbidity, acidification and decreased oxygen concentrations were observed. A nearby rum distillery was identified as a major point sources of pollution, sedimentation and eutrophication in the bay.

FEMS7-2642
Environmental Microbiology/Microbial Ecology /Microbial Communities

TRANSCRIPTOME AND SECRETOME OF SHORT RNAS IN BACTERIAL POPULATIONS

O. Ozoline[1], *A. Bykov*[1], *K. Shavkunov*[1]
[1]*Institute of Cell Biophysics RAS, Functional Genomics and Cellular Stress, Pushchino, Russia*

Backgrounds

Switching research from individual genes to large-scale analysis of transcriptomes revealed a huge amount of untranslated RNAs with poorly understood functions. The least studied are short RNAs, including those processed from tRNAs or produced from gene ends, though their regulatory potential has been recently elucidated.

Objectives

The goal of the study was to characterize the intracellular and extracellular fractions of short (11-14 nucleotides) RNAs, in particularly those derived from “promoter islands” suppressed by H-NS.

Methods

Fractions of short RNAs from cells of *E. coli* K-12 MG1655 and novel isolate of the *Paenibacillus* genus were obtained using mirVana™ miRNA Isolation Kit. Short secreted RNAs were purified from filtered LB medium using miRNeasy Serum/Plasma Kit (Qiagen). cDNA libraries were prepared using Ion Total RNA-Seq Kit v2 and sequenced with Ion Torrent PGM. Sequence reads were mapped to the genomes with Matcher algorithm.

Conclusions

In both bacteria intracellular fractions of short RNAs mainly contained fragments of tRNAs, rRNAs and small untranslated RNAs. The proportion of RNAs derived from the “promoter islands” of *E. coli* was lower than on average over the genome; however, their abundance in the extracellular fraction was twice higher. The distribution profile of the secreted RNAs across the genomes differed, as compared to their intracellular RNAs. Cooperative culturing of two microorganisms allowed detecting evident divergence between the sets of extracellular RNAs in pure and mixed cultures. Bacteria, therefore, secrete not only proteins and small signaling molecules, but also short RNAs, which may participate in cell-to-cell communications.

FEMS7-2295
Environmental Microbiology/Microbial Ecology /Microbial Communities

METAGENOMIC SNAPSHOT: MEASURING PATTERNS BY GEOGRAPHICAL LOCATIONS IN MARINE METAGENOME DATA USING NEWLY ADOPTED GENOTYPING-BY-SEQUENCING

H.J. Seong[1], C.Y. Hwang[2], H.H. Won[3], W.J. Sul[1]
[1]Chung-ang University, Systems Biotechnology, Anseong, Republic of Korea
[2]Korea Polar Research Institute, Division of Polar Biology and Ocean Sciences, Incheon, Republic of Korea
[3]Sungkyunkwan University- Samsung Medical Center,
Samsung Advanced Institute for Health Sciences and Technology, Seoul, Republic of Korea

Backgrounds

Dealing with huge quantity and high complexity of metagenomic data is a very difficult and time-consuming process. In addition, a new metagenomic approach is required to simply identify genetic variation.

Objectives

We demonstrated more effective metagenomic approach to analyze particular sequences related to restriction enzyme sites for targeting reduced numbers of genes than to estimate whole genome sequences.This approach was applied to analyze the marine metagenome by genotyping-by-sequencing (GBS) method, which was used to analyze plant genomes with large genome sizes.

Methods

Metagenomic GBS was demonstrated with marine epipelagic samples collected on 10 locations across from East Sea to Bering approximately 8000 km apart on July, 2013. These samples were processed by a restriction enzyme *Ape*KI and then sequenced by Illumina HiSeq, yielding 74.4 million reads. These restriction site-associated sequences were analyzed to characterize taxonomic diversity and functional profiling of microbial community directly. Moreover, metagenomic GBS reads were grouped, aligned, and Shannon entropy-analysis using UCLUST, MUSCLE, and in-house script from Oligotyping respectively. Entropy in sequence variation among sorted clusters by functional category was measured to evaluate their rate of SNPs variations.

Conclusions

Nucleotide and amino acid substitutions were discovered that indicate micro genetic diversity associated with evolutionary histories according to geographical patterns. Besides profiling microbial community and functional pathway, metagenomic GBS can analyze evolutional study based on nucleotide variation. GBS to metagenomic analysis was applied as a snapshot for understanding the highly dynamic ecosystem quickly to resolve budget and time-consuming issues with identification of micro genetic diversity through comparing each samples.

FEMS7-0773
Environmental Microbiology/Microbial Ecology /Microbial Communities

ESTIMATING MICROBIAL COMMUNITY STRUCTURE USING METABOLIC NETWORKS

K. Takemoto[1]
[1]*Kyushu Institute of Technology, Department of Bioscience and Bioinformatics, Iizuka, Japan*

Backgrounds

Microbes interact with one another via metabolic interactions etc., and they compose ecological communities. It is important to untangle such ecosystems in medical and environmental sciences. In this context, microbial ecological networks are often studied. In general, such networks are estimated based on time-series data on species abundance, obtained from 16S rRNA data, for example. However, such estimation methods have fiscal constraints; moreover, they are not useful for understating microbial ecosystems at the molecular level, despite the importance of metabolite interactions in microbial ecosystems.

Objectives

Thus, I developed a novel method for estimating microbial community structure using metabolic network analysis: Estimator of COmmunity Structure based on MetabOlic networkS (ECOSMOS).

Methods

For this, I considered reverse ecology [R. Levy, E. Borenstein, Adv. Exp. Med. Biol. 751, 329 (2012)]. In particular, cooperative interactions (i.e., complementary support of nutrients) and competitive interactions (i.e., scramble for nutrients) are calculated based on nutrient metabolites identified using graph-theoretic algorithms. In addition to this, I used random matrix theory [S. Suweis et al. Nat. Commun. 6, 10179 (2015)], and evaluated ecological community stability based on the strength of interactions and species abundance.

Conclusions

ECOSMOS considers metabolic interactions between organisms, and it estimates ecological networks from at least one sample. I applied ECOSMOS to actual examples, and found interesting results. For example, the gut microbiota in healthy subjects were highly stable. Cooperation and stability of soil microbial community increase after environmental perturbations. ECOSMOS enhances our understanding of microbial ecological community. ECOSMOS is available from takemoto08.bio.kyutech.ac.jp/ecosmos-lite/

FEMS7-2467
Environmental Microbiology/Microbial Ecology /Microbial Communities

BACTERIAL TRANSCRIPTOME REPROGRAMMING IN NODULES OF PEA (PISUM SATIVUM L.) SYMBIOTIC MUTANTS

V. Zhukov[1], A. Afonin[1], A. Sulima[1], A. Zhernakov[1], I. Tikhonovich[1]
[1]All-Russia Research Institute for Agricultural Microbiology, Biotechnology, Saint-Petersburg - Pushkin, Russia

Backgrounds

Nodule bacteria can fix atmospheric nitrogen in symbiosis with legume plants. In nodules of some legumes, including pea (*Pisum sativum* L.), bacteria are terminally differentiated into organelle-like structures called bacteroids. The process of terminal bacteroid differentiation (TBD) implies cell enlargement, genome amplification and membrane permeabilization. Mutations in plant regulatory symbiotic genes may lead to defects in TBD. Analysis of such model systems using transcriptomics approach can help unravel the molecular bases of TBD.

Objectives

To describe the influence of plant mutations on expression of bacterial genes in case of abnormal TBD and to find possible new factors and components playing a role in this process.

Methods

To assess gene expression levels, we used 5'-MACE (Massive Analysis of cDNA Ends) technology developed by GenXPro GmbH (Frankfurt-am-Main, Germany), which involves sequencing of 5'-part of each transcript on Illumina HiSeq2000. The total RNA was extracted from the following sources: i) free-living culture of *Rhizobium legominosarum* bv. *viciae* RCAM1026, ii) nodules of wild type pea line SGE, iii) set of mutants in pea genes *sym33*, *sym40* and *sym26* obtained on SGE background, which demonstrate block on sequential stages of TBD.

Conclusions

Bacterial genes crucial to cooperative metabolism of micro- and macrosymbiont were identified, along with a number of multi-drug resistance genes possibly modulating the effects of NCR peptides (plant anti-microbial agents promoting TBD) in nodules.

This work was supported by Russian Science Foundation [grant 14-24-00135 for AAM, ZAI, TIA and grant 16-16-00118 for ZVA, SAS].

FEMS7-1407
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

RAMAN MICRO-SPECTROSCOPY BASED QUANTITATIVE ANALYSIS OF POLYPHOSPHATE ACCUMULATING BACTERIA IN FULL-SCALE DANISH WASTEWATER TREATMENT PLANTS

E. Fernando[1], M. Nierychlo[1], S. Mcilroy[1], F.A. Herbst[1], M. Wagner[2], M. Schmid[2], J.L. Nielsen[1], P.H. Nielsen[1]
[1]Center for Microbial Communities- Aalborg University, Chemistry and Bioscience, Aaborg Ø, Denmark
[2]University of Vienna, Department of Microbial Ecology and Ecosystem Science, Vienna, Austria

Backgrounds

Enhanced biological phosphorus removal (EBPR) process is an integral part of wastewater treatment, in terms of removal and recovery of phosphorus from recycled water. The typical polyphosphate accumulating organism (PAO) phenotypes such as the genera Candidatus *Accumulibacter* and *Tetrasphaera* are well-characterised in terms of their P – metabolism taking place under anaerobic/aerobic “feed”/”famine” cycles induced in the EBPR process. The quantitative aspects of phosphorus accumulation in the different PAOs are poorly understood, partly due to shortcomings in currently available quantitative chemical analyses methods.

Objectives

The aim of this work was to establish a Raman spectroscopy-based quantitative method to assess polyphosphate contents within PAO cells.

Methods

This study utilised a non-invasive and non-destructive Raman spectroscopy- based method to quantitatively evaluate intracellular polyphosphate contents of single PAO cells, defined by fluorescence in situ hybridization (FISH), from full-scale Danish EBPR plants.

The method was appropriately calibrated to allow absolute quantification of poly-P amounts within single PAO cells of the populations of Ca. *Accumulibacter* and *Tetrasphaera*. The content was analysed in different plants and in the different aerobic/anaerobic tanks. Most poly-P was accumulated in Ca. *Accumulibacter* cells but since *Tetrasphaera* in general was present in larger abundance, they seemed more important for the removal of P in most plants.

Conclusions

Raman micro-spectroscopy was successfully used to quantitatively characterise single PAO cells from full-scale plants and is very suitable for further studies on the ecology of PAOs.

FEMS7-1891
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ANTIMICROBIAL RESISTANCE PROFILES AFTER CONJUGATIVE EVENTS WITH AN ESCHERICHIA COLI ISOLATE FROM HOSPITAL EFFLUENT

C. Ferreira[1], D. Bogas[1], R. Linheiro[1], A.R. Varela[1,2], O.C. Nunes[2], C.M. Manaia[1]
[1]CBQF - Centro de Biotecnologia e Química Fina,
Universidade Católica Portuguesa- Escola Superior de Biotecnologia, Porto, Portugal
[2]LEPABE, Universidade do Porto- Faculdade de Engenharia, Porto, Portugal

Backgrounds

The dissemination of antibiotic resistance in the environment is an issue of growing concern in which the selection and horizontal gene transfer have important roles. While bacteria of human origin released into sewage already harbour plasmids encoding for antibiotic resistance, further contact with other sewage contaminants may promote the conjugative transfer of resistance traits.

Objectives

This study aims at understanding the influence of candidate stressors on the rate of conjugation as well as characterising the conjugative plasmidome.

Methods

The isolate *Escherichia coli* H1FC54 from hospital effluent was used as donor and the azide resistant *Escherichia coli* J53 as recipient strain. The nucleotide sequence of the conjugative plasmidome obtained with these strains in the presence of ceftazidime was determined. Conjugation assays were carried out in the presence of ceftazidime (10 mg/L), tellurite (0.5-5 µM), or arsenite (0.5-15µ M), and the resistance phenotypes, genotypes and plasmids profiles were determined.

Conclusions

Stressors did not lead to significant increase in the conjugation rates. Transconjugants acquired resistance to amoxicillin, ticarcillin, sulfamethoxazole and tetracycline. Distinct conjugative plasmidomes and variations in the acquired phenotypes were detected in different conjugation assays.
The nucleotide sequence of the conjugative plasmidome revealed the presence of an HI2 plasmid backbone with determinants of resistance to antibiotics (beta-lactams, (fluoro)quinolones, sulfonamides), tellurite and arsenite.
The variable composition of the conjugative plasmidome and the formation of cointegrates during conjugation are novel findings, which may bring interesting insights into the comprehension of the molecular and physiological mechanisms that underlie antibiotic resistance propagation in the environment.

FEMS7-0624
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EXTENDED-SPECTRUM-B-LACTAMASE PRODUCING ESCHERICHIA COLI IN HEALTHY UNIVERSITY STUDENTS OF PORTO PORTUGAL

R. Mota[1], M. Pinto[2], J. Palmeira[3], D. Gonçalves[4], H.M.N. Ferreira[5]
[1]University of Porto, Microbiology - Biological Sciences - Pharmacy Faculty, Porto, Portugal
[2]University of Porto, Microbiology- Biological Sciences - Pharmacy Faculty, Porto, Portugal
[3]University of Porto, Micribiology - UCIBIO - Pharmacy Faculty, Porto, Portugal
[4]University of Porto, Microbiology- UCIBIO- Pharmacy Faculty, Porto, Portugal
[5]University of Porto, Porto, Portugal

Backgrounds

Extended-spectrum-β-lactamases (ESBLs) are increasingly being reported colonizing humans. Antimicrobial resistance mechanisms are spreading not only in healthcare institutions but also in community.

Objectives

Our aim was the detection of ESBL and carbapenemase-producing *Enterobacteriaceae* in healthy young-adults of University of Porto.

Methods

Faecal samples of 30 students were analyzed in MacConkey agar with cefotaxime, ceftazidime and meropenem (2 mg/L). Antimicrobial susceptibility of representatives of different colony morphologies was studied according to the CLSI. Bacterial identification was performed by CHROMagar-orientation, API20E and ID32GN, and ESBL-producers were detected and/or confirmed by the double-disk-synergy-test and clavulanic-acid addition. Detection of *bla*$_{\text{TEM}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{CTX-M-group-1/group-2/group-8/group-9/CTX-M-group-25}}$ and *tetA*-B, was performed by PCR. In isolates showing susceptibility reduction to carbapenems we searched for prevalent carbapenemases. In ESBL-producers, we searched for virulence factors and phylogenetic-groups. We addressed transferability of resistance and virulence genes by conjugation. Students colonized with ESBL-producers were followed up after 5 months.

Conclusions

Our study showed the presence of ESBL-producing-*Enterobacteriaeceae* in healthy young-adults. ESBL-producing *E. coli* were isolated from 2 students (6.7%). These isolates showed *bla*$_{CTX-M}$-group1 (n=1), *bla*$_{CTX-M}$-group9 (n=2), *bla*$_{TEM}$ (n=2), *bla*$_{SHV}$ (n=1) and *tetA* (n=2) genes, and specific virulence-factors *fimH* (n=3), *traT (*n=3), *fyuA* (n=2), *iutA* (n=2) and *cvaC* (n=1). Transconjugants acquired resistance and virulence genes. Carbapenem resistant *Pseudomonas* spp was detected colonizing one student. One of the students colonized with ESBL-producer remained colonized after 5 months. Results show that healthy young-adults can be a reservoir of ESBL-producers and that *E. coli* is the ESBL-producing species most often found in community, suggesting silent dissemination.

FEMS7-2722
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

STUDYING BIOFILM FORMATION DYNAMICS IN REAL-TIME

M.D. Ferrer[1], J.C. Rodriguez[2], E. Buetas[1], C. Collantes[1], I. Vidal[2], A. Mira[1]
[1]Center for Advanced Research in Public Health, Genomics and Health Area, Valencia, Spain
[2]Hospital General Universitario de Alicante Spain, Microbiology, Alicante, Spain

Backgrounds

Several methods are available to study biofilms *in vitro*, but their applications are in many occasions limited by a low sensitivity, high labor intensity and/or long time lags to obtaining a result. In addition, a generalized weakness is the fact that results are observed only at a certain time ("end-point" methods), with the consequent loss of information.

Objectives

The aim of this study is to present and evaluate a reliable, labeling-free and rapid assay for quantifying mono- and multi-species biofilm formation in real time.

Methods

We performed impedance-based technology measurements in microtiter plates with gold electrodes using RTCA xCELLigence system (ACEA Biosciences).

Conclusions

Biofilm growth curves showed an S-shape as cells grew attached to the wells disrupting the electrical current. The effect of 10 antibiotics on biofilm formation dynamics of different clinical strains of *Staphylococcus aureus*, *S. epidermidis* and *Pseudomonas aeruginosa* has been studied. Antibiotic resistance patterns in biofilms were very different to those obtained by traditional methods. Complex biofilm formation of oral samples (saliva, tongue and plaque) have also been monitored. Biofilm formed was a multi-species biofilm of similar composition and abundance to the original inoculum and the patterns of biofilm growth were different between them. In summary, RTCA is able to quantify mono- and multi-species biofilms in real time. We propose the use of real-time measurements for shedding light on the biology of mono- and multi-species biofilm formation, including diseases of polymicrobial etiology, and for clinical applications like the selection of efficient antibiotic therapy in biofilm-forming pathogens.

FEMS7-1238
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EVIDENCE OF HELICOBACTER HEPATICUS PRESENCE IN WASTEWATER BY METAGENOMICS

I. Hortelano[1], Y. Moreno[1], R. Pérez-Santonja[1], M.A. Ferrús[2]
[1]Universitat Politècnica de València, Research Institute of Water Engineering and Environment IIAMA, Valencia, Spain
[2]Universitat Politècnica de València, Biotecnología, Valencia, Spain

Backgrounds

Non-*H. pylori Helicobacter* (NHPH) species produce gastric diseases in human, including gastritis and mucosa-associated lymphoid tissue (MALT) lymphoma.

H. hepaticus is the most studied NHPH specie, as it is related with liver and biliary tract diseases. Human intestine seems to be its primary colonization site; therefore, it could be present in fecal-contaminated water. *In vitro* isolation of NHPH is extremely difficult and this specie has never been isolated from environmental samples. Thus, only molecular culture-independent methods can be successful for detection.

Objectives

We have investigated the occurrence of *H. pylori* and NHPH in wastewater by genus specific PCR targeting 16S-RNA gene. Furthermore, a 16S rDNA -base metagenomics approach was developed.

Methods

Eight samples from the tertiary treatment effluent from a wastewater treatment plant were processed. DNA was amplified, submitted to genus specific PCR and subsequently sequenced. Positive samples were analyzed by metagenomics: the hypervariable V1-V3 region of the 16S rDNA was amplified and deep sequenced using an Illumina MiSeq platform. The analysis of the sequences was performed using the QIIME software.

Conclusions

Results obtained showed that 6 of the analyzed samples were positive for *Helicobacter* genus. After sequencing, two of the samples were positive for *H.pylori* and other two were positive for *H. hepaticus.* These results were verified by metagenomics analysis. Since our knowledge, this is the first time that *H. hepaticus* has been identified from wastewater.

This study has been supported by the Spanish Ministerio de Economía y Competitividad AGL2014/53875-R grant

FEMS7-1783
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EXPLORING THE INFLUENCE OF GEOGRAPHY AND THALLI LOCATION ON BACTERIAL COMMUNITIES ASSOCIATED WITH THE LICHEN RAMALINA FARINACEA

À. Figàs-Segura[1,2], E. G. Biosca[1], E. Barreno[2,3], N. Fierer[4,5]
[1]Universitat de València, Microbiología y Ecología, Burjassot, Spain
[2]Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Botánica, Paterna, Spain
[3]Universitat de València, Botánica, Burjassot, Spain
[4]University of Boulder, Department of Ecology and Evolutionary Biology, Boulder- CO, USA
[5]University of Boulder, Cooperative Institute for Research in Environmental Sciences, Boulder- CO, USA

Backgrounds

The classical definition of lichen comprises the mycobiont (a fungus) and the photobionts (green algae and/or cyanobacteria) as the symbiotic mutualistic partners. However, recent studies have demonstrated that heterotrophic bacterial communities are additional partners, with lichens now considered as multi-species symbiosis. Nevertheless, still very little is known about the composition and diversity of these communities, and how they could vary across geography and/or the lichen structure.

Objectives

We aimed to explore the diversity and composition of the bacterial communities associated with populations of the lichen *Ramalina farinacea* from different geographical locations, as well as between ectolichenic and endolichenic fractions and along differentiated thallus parts.

Methods

Lichen thalli of *R. farinacea* from four different locations in Spain with Mediterranean climate, two in the Canary Islands and two in the Iberian Peninsula, were analyzed by multiplexed sequencing of the V4-V5 region of the *16S rRNA* gene.

Conclusions

Our results provide new insights into the diversity of bacterial communities associated with *R. farinacea* populations from different Mediterranean-climate areas, showing that the predominant phylum at the four locations was the *Proteobacteria,* particularly *Alphaproteobacteria*, the families *Acetobacteriaceae, Caulobacteriaceae and Methylocystaceae* and the genera *Beijerinckia* and *Sphingomonas* among others. Furthermore, the bacterial community composition appears mainly determined by geography since some taxa within the *Cyanobacteria* and *Firmicutes* phyla were more abundant in the *R. farinacea* populations from the Island than in those from the Peninsula. Also, the communities were distinct between ectolichenic and endolichenic fractions and among apical, middle and basal thallus parts. (PROMETEOII/2013/021 & VALi+d-BEFPI; BACTPLANT-UV-Research Support; FiererLab).

FEMS7-3108
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COMPARATIVE GENOMIC ANALYSIS OF FAECALIBACTERIUM PRAUSNITZII STRAINS ISOLATED FROM HEALTHY HUMAN GUT

C.B. Fitzgerald[1], A. Shkoporov[1], V. Velayudhan[1], A. Forde[1], C. Hill[1], P. Ross[1]
[1]APC Microbiome Institute, Microbiology, Cork, Ireland

Backgrounds

Faecalibacterium prausnitzii is a member of the *Ruminococcaceae* family and one of the most predominant members of human gut microbiome. *F. prausnitzii* has been shown to decrease significantly in patients with IBD. It has also been shown to possess direct anti-inflammatory activities in mouse colitis models. Despite its predominance and positive role in the healthy human gut, little is known about it's genomic organization, genetic diversity, and the phylogenetic links of this species with related genera inhabiting the human gut.

Objectives

The objective of this project was to perform complete/draft genomic sequencing of 12 *F. prausnitzii* strains and one closely related *Subdoligranulum/Gemmiger* strain. These strains were isolated from healthy human subjects and were analysed comparatively in conjunction with 10 previously sequenced genomes available from public databases.

Methods

A total of 12 strains of *F. prausnitzii* were isolated from anaerobic cultures of healthy human fecal samples. These strains were identified by a combination of phenotypic tests and 16S rRNA gene sequencing. Draft genome sequencing of all strains was accomplished using Illumina Hiseq platform. Two strains were also subjected to PacBio sequencing for gap closing. An array of bioinformatic and molecular techniques were used to identify core genome composition, phylogenetic relatedness of *F. prausnitzii* in context of the family *Ruminococcaceae*, mobilome, and other genetic features.

Conclusions

Preliminary analysis results suggest that human *F. prausnitzii* isolates demonstrate high level of genetic polymorphism and genomic shuffling. Plasmids, and inducible prophages were identified. The results from this project will greatly increase our understanding of *F. prausnitzii.*

FEMS7-0677
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

RHIZOBIA BIOFERTILIZER-BASED INCREASE YIELD PRODUCTION IN CARROTS

J.D. Flores Felix[1], P. García-Fraile[2], E. Martínez-Molina[1], E. Velázquez[1], R. Rivas[1]
[1]University of Salamanca, Microbiología y Genética, Salamanca, Spain
[2]Academy of Science of the Czech Republic, Department of Microbiology, Prague, Czech Republic

Backgrounds

Modern agriculture has to solve the problem of feeding an increasing population in a World with limited resources. Dealing with this challenge requires the application of new technologies in the fields to improve the efficiency of the available resources. One example is the application of biofertilizers formulated with Plant Growth Promoting Rhizobacteria (PGPR).

Objectives

The aim of this study was to develop a bacterial biofertilizer for carrots with the capability to promote this crop yields under field conditions.

Methods

We selected the strain PEPV16, a bacterium with several PGPR mechanism (Flores-Félix et al., 2015). Then, we checked the capability of the GFP-labelled strain to colonize carrots' root surface, using a fluorescence microscope. To study the capability of the bacterial strain to promote the first plant stages, inoculated seeds were cultivated in water-agar plates overlaid with Whatman number 1 sterile paper. After 7 and 14 days, stem and root length was measured and the number of secondary root was counted. Field experiments were performed and statistical analyses using StatView program allowed us to compared the carrots production from plant inoculated with the strain PEPV16 and the positive (chemical fertilizer) and the negative controls.

Conclusions

The strain PEPV16 is able to produce indolacetic acid-like compounds, siderophores and solubilize phosphates. It increases inoculated seedlings aerial and root length, and the number of secondary roots. In field conditions, inoculated carrots show an increase in root weight, which produces an improvement in caliber of yielded carrots, and reduce the malformation in roots.

FEMS7-3094
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE PLANT-BENEFICIAL ROOT-COLONIZER PSEUDOMONAS PROTEGENS CHA0 PERSISTS IN INSECTS THROUGHOUT DIFFERENT DEVELOPMENTAL STAGES AND CAN BE TRANSMITTED TO NEW HOST PLANTS

P. Flury[1,2], P. Vesga[1], A. Dominguez-Ferreras[1], D. Bär[1], C. Tinguely[1], J. Schneider[1], A. Taddei[1], R. Kleespies[3], C. Keel[2], M. Maurhofer[1]
[1]ETH Zürich, Phytopathology, Zürich, Switzerland
[2]University of Lausanne, Department of Fundamental Microbiology, Lausanne, Switzerland
[3]Julius Kühn-Institute JKI,
Federal Research Centre for Cultivated Plants- Institute for Biological Control, Darmstadt, Germany

Backgrounds

The discovery of insecticidal activity in root-colonizing pseudomonads, best-known for their plant-beneficial effects, raised not only questions about a possible application of this trait in pest control, but also about the ecological relevance of insects as alternative hosts for these bacteria. Since soil bacteria are limited in their inherent abilities of dispersal, insects as vectors might be welcome vehicles to overcome large distances.

Objectives

This study investigates whether *Pseudomonas protegens* CHA0 exhibits biocontrol activity against a root-feeding insect pest and whether insects could serve as a vector for dispersal of this plant-beneficial rhizobacterium.

Methods

In a pot experiment larvae of the cabbage fly were feeding on roots colonized by *P. protegens* CHA0-*gfp2*. Emerging adult flies were exposed to new, bacteria free plants, imitating the natural situation in which flies search for new host plants to deposit eggs. Bacterial numbers (in roots and insects) and insect survival were monitored. Persistence of *P. protegens* CHA0 in insects throughout different life-stages was further assessed in additional insect species and investigated by microscopy methods.

Conclusions

P. protegens CHA0 is able to persist in several insect species throughout different life-stages and can be transmitted from the roots of one plant to the roots of another plant by the cabbage root fly. Although, survival of the latter was not affected by CHA0, morphological defects observed in parts of adult flies indicate a negative impact of the bacteria on insect development. In summary, our results suggest that certain fluorescent pseudomonads can use insects as hosts and vectors.

FEMS7-0824
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DISULFIDE BOND-CONTAINING AJOENE ANALOGS AS NOVEL QUORUM SENSING INHIBITORS OF PSEUDOMONAS AERUGINOSA

J. Fong[1,2], T.H. Jakobsen[3], K.T. Mortensen[4], M.M. Salido Delos Santos[1], S.L. Chua[1], Y. Liang[1,2], C.H. Tan[5], T.E. Nielsen[1,3], M. Givskov[1,3]
[1]Singapore Centre for Environmental Life Sciences Engineering, Public Health and Medical Biofilm, Singapore, Singapore
[2]Nanyang Technological University, School of Biological Sciences, Singapore, Singapore
[3]University of Copenhagen, Costerton Biofilm Center- Department of Immunology and Microbiology, Copenhagen, Denmark
[4]Technical University of Denmark, Department of Chemistry, Copenhagen, Denmark
[5]Nanyang Technological University,
Chemistry and Biological Chemistry- School of Physical and Mathematical Sciences, Singapore, Singapore

Backgrounds

Infectious diseases are the direct cause of 16 million deaths annually. Among those, estimated 65% fatalities are associated with biofilm infections. QS inhibitors (QSI) have been proposed as a promising agent for controlling persistent infections, where deletion of one or more QS genes in *P. aeruginosa* result in lower virulence factors as compared to the wild type and render biofilm bacteria more susceptible towards antibiotic treatment.

Objectives

Identify and develop novel chemical scaffolds as potential quorum sensing inhibitors (QSIs) and antivirulence drugs for treatment of *P. aeruginosa* infections.

Methods

We performed screening of in-house chemical library and structural activity relationship (SAR) study to improve bioactivities to sub-micromolar range. The compounds were then tested against our QS bioreporter strains (*lasB, rhlA, pqsA-gfp*), as well as on QS-activated virulence phenotypes such as elastase, rhamnolipid and pyocyanin production. Lastly, we also tested the efficacy of compounds *in vivo* using mouse implant biofilm infection model.

Conclusions

Our SAR study indicated a novel benzothiazole derivative with IC_{50} value of 0.56 µM, which is one of the most potent QS inhibitors reported to date. The compounds were also able to reduce expression of QS-regulated virulence factors and clear *P. aeruginosa* infection in a mouse implant biofilm infection model. Altogether, the QSI activity of the synthesized compounds is encouraging for the further exploration of novel analogs in antimicrobial drug development. The results presented here, from high-throughput screening (HTS) to SAR studies, could provide important scaffolds for future chemical development, in both design and discovery of novel QSIs.

FEMS7-0570
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE MICROBIAL ECOLOGY AND BIOGEOCHEMISTRY OF A NUCLEAR FUEL STORAGE POND

L. Foster[1], J. Pittman[1], K. Morris[1], D. Sigee[1], H. Muhamad Ali[2], R. Goodacre[2], J. Lloyd[1]
[1]University of Manchester, School of Earth and Environmental Sciences, Manchester, United Kingdom
[2]University of Manchester, Manchester Institute of Biotechnology, Manchester, United Kingdom

Backgrounds

The Sellafield site has several open spent nuclear storage ponds (SNFP) that are at various stages of decommissioning, including an alkaline pond containing spent fuel and sludge waste, which are to be retrieved. This pond is extremely inhospitable with significant levels of radioactivity and high pH (~11) due to the continuous purging of highly alkaline water. Despite these conditions the pond is known to experience seasonal microbial growth, described as “algal blooms”. The blooms reduce visibility in the pond, impacting on waste retrieval operations and potentially increasing both the cost and timescale of hazard and risk reduction. Currently there is little information about the organisms responsible for the blooms, what the triggers for the bloom are, or how the microorganisms colonise such an extreme environment.

Objectives

The aim of this project is to characterise the microbial community residing in this pond. Initial analyses of DNA extracted from the pond water samples revealed the presence of a cyanobacterium, *Pseudanabaena catenata*.

Methods

Cultures of *P. catenata* were exposed to 95 Gy of X-irradiation over a 5 day period to monitor the effect that ionizing radiation had on the growth of the organism. Cell pellets were analysed using FTIR to get a fingerprint of the metabolic activity in the irradiated cells.

Conclusions

P. catenata showed no change in growth yield when irradiated. However the cultures did show a significant reduction in the chlorophyll concentrations following the treatment. FTIR results indicate an increase in polysaccharides, which may significantly impact on the fate of radionuclides in the pond.

FEMS7-1577
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL DIVERSITY IN EXTREME SALINE ENVIROMENTS

M. Franze[1], *A. Cherkouk*[1]
[1]*HZDR, Institute of Resource Ecology - Biogeochemistry, Dresden, Germany*

Backgrounds

Rock salt formations are considered as potential host rock systems for the long-term storage of highly radioactive waste in a deep geological repository. To date, little is known about the habitat rock salt and the way of life of the microorganisms occurring there. Next to bacteria and fungi, extreme halophilic archaea are dominating this habitat. It is of interest to know what kind of microorganisms are living there, how active they are under repository relevant conditions and how these microorganisms can influence the safe storage of the waste.

Objectives

A combination of culture-dependent and -independent methods was used to investigate the microbial diversity in rock salt from potential host rock for nuclear waste disposal as well as saline soil samples from Arava Desert, Israel.

Methods

Culture-dependent: A specific portion of the two kinds of samples were incubated in three different sodium chloride concentrations of modified R2A resuscitation buffer and were spread on corresponding agar plates (37°C) to get isolates which were further characterized. Culture-independent: From two samples DNA was extracted, purified for PCR amplification of 16S rRNA genes and sequenced with Illumina MiSeq (RTL Genomics).

Conclusions

Halophilic microorganisms could be isolated from both kinds of samples. The soil sample isolates can be assigned to different archaeal genera *Natrinema*, *Halorubrum*, and *Halobacterium.* Bacterial isolates could be related to *Bacilli* such as *Halobacillus* and *Aquibacillus*. From rock salt samples different *Halobacetrium* species could be isolated. The obtained isolates could be further used for investigations, regarding their activity under repository relevant conditions.

FEMS7-2426
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ECOINFORMATIC DECODING OF THE RABBIT MICROBIOME

G. Funosas[1], F. Castro[2], R. Villafuerte[2], E. O. Casamayor[1], X. Triadó-Margarit[1]
[1]Centre of Advanced Studies of Blanes-Spanish Council for Research CEAB-CSIC, Integrative Freshwater Ecology Group, Blanes, Spain
[2]Institute of Advanced Social Studies IESA-CSIC, TRAMAS - Territorio- Ruralidad- Medio Ambiente y Sostenibilidad, Córdoba, Spain

Backgrounds

The gut microbiome is a current hot topic of research, not only directly related to diet and nutritional status studies but also because the close relationships with physiological and immunological responses in animals, proving to be a key factor in the host health status. We analyzed the gut microbiome in the wild European rabbit (*Oryctolagus cuniculus*), a keystone species for Iberian biodiversity,

Objectives

We aimed to determine the identity and structure of the gut microbiome and the factors that shape its composition. We hypothesized that the proportion of different microbial groups can be used as a proxy for host health status.

Methods

We applied high-throughput sequencing Illumina techniques (16S rRNA amplicon) to fecal rabbit samples. Factors such as captivity, diet, age, sex, host taxonomy and other biometric variables were analyzed. A set of healthy domestic rabbits was used to validate the health index.

Conclusions

Only diet and captivity status significantly shaped the microbiome composition. The highest diversity was found in wild rabbit whereas in many captive animals a consistent dominance by Escherichia-Shigella was observed. A significant negative correlation between relative abundances of Ruminococcaceae-Lachnospiraceae-Rikenellaceae and Enterobacteriaceae was found. The rabbit gut microbiome composition varied accordingly to the health status of the animals unveiling the ratio Ruminococcaceae/Enterobacteriaceae as a key parameter for healthy individuals.

FEMS7-1698
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL GRANULATION AT HIGH SALINITY: A STRESS INDUCED SURVIVAL STRATEGY?

M.C. Gagliano[1], T. Neu[2], U. Kuhlicke[2], D. Sudmalis[3], H. Temmink[3], C. Plugge[1]
[1]Wageningen University, Laboratory of Microbiology, Wageningen, The Netherlands
[2]Helmholtz Centre for Environmental Research UFZ, Department of River Ecology, Magdeburg, Germany
[3]Wageningen University, Sub-department of Environmental Technology, Wageningen, The Netherlands

Backgrounds

In the Upflow Anaerobic Sludge Blanket Reactor (UASB) technology, microorganisms auto-aggregate as a granular biofilm, forming a microbial ecosystem with a characteristic architecture. When saline wastewater is biologically treated in this system, the sodium concentration can negatively affect microbial aggregation and consequently the anaerobic process. On the other hand, microbial aggregation, with the production of extracellular polymeric substance (EPS), is described as a protective response to environmental stress. In this research, we analysed the structure of anaerobic granules in two UASB reactors treating low (5 g/L of sodium) and high (20 g/L of sodium) salinity synthetic wastewater.

Objectives

Identify granules components (extracellular polymers, microorganisms) to clarify the granule formation mechanism at low and high salinity, pointing out the role of the main microbial protagonists.

Methods

Fluorescence lectinbar-coding (FLBC) analysis coupled with Confocal Laser Scanning Microscopy (CLSM) revealed a completely different glycoconjugate composition at two different salinities. Fluorescence in Situ Hybridization (FISH), 16S rRNA analysis and Scanning Electron Microscopy (SEM) highlighted that the non-halophilic *Methanosaeta harundinacea spp.* was the dominant methanogen in both granules, constituting the main “builiding block” of the structure. Conversely, a completely different bacterial community between the low and high salinity reactors had developed.

Conclusions

Halotolerance in UASB reactors is facilitated by means of EPS/biofilm production. EPS glycoconjugate distribution and the ratio between the components in granules were influenced by the different salinity levels and the different microbial community members.

FEMS7-2301
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE SEED ENDOSPHYTIC MICROBIOTA OF CITRUS LIMON L. BURM. F.

T. Faddetta[1], L. Abbate[2], I. La Mendola[1], C. Maragliano[1], P. Alibrandi[1], F. Strati[3], C. De Filippo[4], F. Carimi[2], M. Cardinale[5], G. Gallo[1], F. Mercati[2], A.M. Puglia[1]
[1]University of Palermo, STEBICEF, Palermo, Italy
[2]National Research Council of Italy CNR, Institute of Biosciences and Bioresources IBBR, Palermo, Italy
[3]Research and Innovation Centre- Fondazione Edmund Mach, Computational Biology Research Unit, San Michele all' Adige, Italy
[4]National Research Council of Italy CNR, Institute of Agricultural Biology and Biotechnology, Pisa, Italy
[5]Justus-Liebig-University Giessen, Institute of Applied Microbiology, Giessen, Germany

Backgrounds

Plant seeds possess a complex microbiota which may play a crucial role in many aspects, such as preservation, germination, seedling development, plant growth and health. In particular, seed endophytic microbiota is gaining more and more consideration due to the fact that it may be vertically transmitted to ensure individual competitive advantages. The *Citrus* species, including *C. limon*, are one of the most economically important evergreen fruit crops in the world.

Objectives

i) Identification and characterization of microbial isolates from *Citrus limon* L. Burm. F. seed endosphere.
ii) Structure of *Citrus limon* L. Burm. F. seed endosphytic microbiota.

Methods

- Surface-sterilization of *Citrus limon* L. Burm. F. seeds.
- Strain isolation and phylogenetic characterization by 16S rDNA sequence.
- Next Generation Sequence (NGS) technology analysis of metagenomic DNA by pyrosequencing of 16S rDNA.
- Fluorescence In Situ Hybridization coupled with Confocal Laser Scanning Microscopy (FISH-CLSM).

Conclusions

Culture-dependent approaches allowed the isolation of several bacterial strains belonging to the genus *Staphylococcus* and several fungal strains belonging to the genera *Aspergillus, Quambalaria and Efibula* from seed endosphere. These results were supported by the detection of bacterial cells and micro–colonies in seed cryosections by FISH-CLSM. In particular, this analysis highlighted the presence of Firmicutes and other bacteria colonizing intercellular spaces. In addition, NGS-based characterization using metagenomic DNA from seed endosphere is being carried out in order to elucidate the microbiota structure.

FEMS7-2418
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ANALYSIS OF MALDI-TOF MS PROFILES OF ISOLATES RECOVERED FROM DIFFERENT DRINKING WATER SOURCES

B. Galofré[1], G. Saucedo[1], C. Vilaró[1], S. Fernández[2], D. Baquero[2], A.R. Blanch[3], F. Lucena[3], C. García-Aljaro[3], L. Sala-Comorera[3], D. Toribio[3], M.A. Ruvira[4], L. Rodrigo-Torres[4], M.C. Macián[4], D.R. Arahal[4], M.J. Pujalte[4], R. Aznar[4]
[1]Aigües de Barcelona- Empresa Metropolitana de Gestió del Cicle Integral de l'Aigua- SA, Direcció Qualitat Aigua - Laboratori de Microbiologia, Barcelona, Spain
[2]Cetaqua- Water Technology Center, Quality area, Barcelona, Spain
[3]Faculty of Biology. University of Barcelona, Department of Genetics- Microbiology and Statistics, Barcelona, Spain
[4]Spanish Type Culture Collection CECT, Department of Microbiology and Ecology, Valencia, Spain

Backgrounds

Rapid identification of bacteria can be approached by molecular techniques as an alternative to traditional culture methods. MALDI-TOF MS (matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry) has proven as an easy-to-use, efficient, reliable and fast technique in routine clinical analysis. However, the commercial database for identification of environmental bacteria in drinking water laboratories is still very limited by the lack of a suitable mass spectra database. This work is part of the on-going project *Drinking Water Library*, which aims to generate a MALDI-TOF MS database useful for water laboratories and water treatment - distribution operators.

Objectives

Evaluation of the MALDI-TOF profiles for the identification of drinking water associated bacteria using the current commercial database.

Methods

Isolation of bacteria from bottled mineral water, mineral source water, drinking water treatment plant (DWTP) and distribution network by plating on R2A Agar and Water ISO media followed by incubation at 22ºC for up to 7 days. Isolates identification by MALDI-TOF using the current database. Profiles clustering of unidentified isolates and selection of cluster representatives.

Conclusions

A total of 1014 strains have been isolated and processed so far from bottled mineral water (307), mineral source water (349) and DWTP and distribution network (358). 44% of the isolates were identified at species level as belonging to 36 different genera, being the majority *Bacillus* and *Pseudomonas*. 56% remain unidentified at species level and clustered in 22 profile groups. Identification of cluster representatives will lead the selection of species to be included in the Drinking Water Library.

FEMS7-1609
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TAXONOMIC AND FUNCTIONAL DIVERSITY OF PHYLLOSPHERIC COMMUNITIES IN AN URBAN PARK (MILAN, NORTHERN ITALY)

I. Gandolfi[1], I. Rossi[1], S. Molinetti[1], D. Brambilla[1], S. Covino[2], R. Ambrosini[1], E. Padoa-Schioppa[1], M. Papacchini[3], D. Cappelletti[2], E. Federici[2], A. Franzetti[1]
[1]University of Milano-Bicocca, Dept. of Earth and Environmental Sciences, Milan, Italy
[2]Università degli Studi di Perugia, Dipartimento Di Chimica- Biologia e Biotecnologie, Perugia, Italy
[3]INAIL,
Dipartimento Innovazioni Tecnologiche e Sicurezza degli Impianti- Prodotti ed Insediamenti Antropici, Rome, Italy

Backgrounds

Plant-associated microorganisms have been suggested to play a role in air pollution mitigation, especially in urban areas. Particularly, epiphytic bacteria and fungi might be able to degrade atmospheric hydrocarbons. However, phyllospheric microbial communities are highly variable depending on several factors, e.g. tree species, leaf age, environmental conditions.

Objectives

In this work, temporal variability of epiphytic bacterial and fungal communities hosted by leaves of different tree species (*Magnolia grandiflora, Cedrus deodara, Platanus x acerifolia, Tilia x vulgaris)*, located in an urban park in Milan (northern Italy), was assessed during a year-long sampling campaign.

Methods

Bacterial and fungal communities were taxonomically characterized by Illumina high throughput sequencing of the hypervariable regions V5-V6 of 16S rRNA gene and of the ITS1-ITS2 regions, respectively. Functional biodiversity was assessed by whole metagenomic sequencing on a subset of samples. Culturable bacterial strains, both epiphytic and endophytic, were isolated on oligotrophic media and tested for biodegradation abilities towards PAHs.

Conclusions

Results revealed that each tree species hosted peculiar microbial populations. Temporal changes, which are related to seasonality, acted as a strong driver on biodiversity of microbial communities. Moreover, temporal differences were more marked in deciduous plants than in perennial plants. Whole metagenomic analyses indicated the presence of aromatic hydrocarbon degrading abilities, although at low coverages. Approximatively 7% of the isolated strains were able to degrade naphthalene and/or phenanthrene in laboratory cultures. It was therefore hypothesized that phyllospheric populations hosted by urban trees might play a role in the mitigation of hydrocarbon air pollution.

FEMS7-2959
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MULTIPLE QUORUM-SENSING SIGNALS PRODUCED BY NITROBACTER N-ACYL-HOMOSERINE LACTONE SYNTHASE NWII

J. Gao[1], G. Zhuang[1]
[1]Research Center for Eco-Environmental Sciences- Chinese Academy of Sciences, CAS Key Laboratory of Environmental Biotechnology, Beijing, China

Backgrounds

The genome sequence showed that the nwi_0626 gene in *Nitrobacter winogradskyi* was predicted to encode LuxI homolog that is responsible for the synthesis of acyl-homoserine lactones (AHLs) as quorum sensing (QS) signals. Although the AHL-producing activity was confirmed in *N. winogradskyi*, the AHL synthase, LuxI/R QS system and the postulated QS in *N. winogradskyi* have not been proven.

Objectives

To investigate the potential AHL synthase from *N. winogradskyi*, we sought to identify the signal types that were produced by NwiI.

Methods

The *nwiI* gene of *N. winogradskyi* was confirmed to be a homoserine lactone synthase by heterologous expression in *Escherichia coli,* and we purified a novel AHL molecule with an unsaturated C10 acyl side chain generated by NwiI, as confirmed by the mass, nuclear magnetic resonance (NMR) and correlation spectroscopy (COSY) analyses.

Conclusions

To identify the signal types that were produced by the AHLs synthase in *N. winogradskyi*, we introduced *nwiI* into *E. coli*, which resulted in the synthesis of a series of AHLs with chain lengths ranging from C7 to C11. Five of the AHLs were identified as C7-AHL, C8-AHL, C9-AHL, C10-AHL and C10:1-AHL. A novel signal, 7, 8-*trans*-*N*-(decanoyl) homoserine lactone (C10:1-AHL), was identified in both *N. winogradskyi* and the recombined *E. coli*. Furthermore, this novel signal also triggered variances in the nitrification rate and the level of transcripts for the genes involved in the nitrification process. These results indicate that quorum sensing may have a potential role in regulating nitrogen metabolism.

FEMS7-1983
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE GOLLUM PROJECT: CHARACTERISING SUBTERRANEAN BACTERIAL COMMUNITIES IN DEPTH(S)

C.P. Garay[1]
[1]*Institute for Integrative Systems Biology I2SysBio, Theory, Paterna, Spain*

Backgrounds

The Somport tunnel (connecting Spain and France in central Pyrenees) crosses different rocks from the late Paleozoic ages, and includes several Facies. Its length, depth and diverse ecology make it a perfect site for extremophile ecology studies. In extreme environments, bacteria –and archaea- tend to be the main living organisms. Subterranean microorganisms have been described, but almost all reports refer to samples taken centimeters to few meters below the surface. In fact, many of those have a photoautotrophic metabolism. By contrast, the literature describing microorganism inhabiting the very inside of rocks are scarce. The few reports analysing the microbial diversity of rock inhabitants evidence, though, a rather high diversity of microbial taxa and metabolism pathways.

Objectives

Gollum goal is the identification and characterization of the microbial communities living in a range of different rocks throughout the length of the Somport tunnel, from the surface to the maximum depth (850 meters).

Methods

This is being accomplished through geochemical analysis and 16S amplicon and shotgun high throughput sequencing of the combined genomes in a given sample. Sampling different depths and rocks is achieved by collecting one-meter length cylinders of rock drilled along the tunnel, minimizing external contamination.

Conclusions

Taken together, these procedures allow determining with high precision the microbial composition of the Somport tunnel at different depths and on different mineral substrates. This pioneering study may serve as a platform for further geomicrobiology (e.g., biology) studies in already existing deep underground labs, nowadays mainly dedicated to low background physics experiments.

FEMS7-1398
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

NEW MICROBIAL SOURCE TRACKING MARKER BASED ON THE PREDICTED BACTERIOPHAGE CRASS GENOME

C. García-Aljaro[1], E. Ballesté[1], M. Muniesa[1], J. Jofre[1]
[1]University of Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain

Backgrounds

In the last decades considerable efforts have been devoted to the search of microbial source tracking markers in order to predict the associated health risks of fecally polluted waters, with no universal marker reported so far. A recent metagenomic study identified the genome of a bacteriophage referred to as crAssphage which was abundant and widespread in human faecal samples.

Objectives

To develop a quantification method to study the real abundance and prevalence of crAssphage in wastewaters of human and animal origins.

Methods

The presence of crAssphage was investigated in the DNA extracted from raw samples (total and viral DNA fraction) by qPCR using a TaqMan® assay. Primers and probe were designed from a 1331 bp fragment of the sequence of crAssphage (NC_024711.1) from a human sewage sample.

Conclusions

CrAssphage showed up to 10^8 GC/100 ml in all sewage samples from human origin in the total DNA and in the viral fraction. In animal wastewaters, 36% of the samples were negative for the presence of the phage and those showing positive results were between 3 and 2 log10 units (36%) lower than samples from human origin; the remaining showed 1 log10 units difference. The ratios between the numbers of crAssphage and indicators of general fecal contamination were higher in samples with human fecal contaminants. This study opens the door for further research to explore if different specific animal variants of crAssphage exist and whether other zones of the genome of crAssphage have better capability of discrimination of the source.

FEMS7-2501
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COMMUNITY DIVERSITY AND NICHE STRUCTURES AFFECT DIFFERENTLY THE EVOLUTION OF SECRETED PROTEINS TO MAXIMIZE COMPETITION

M. Garcia-Garcera[1], *M. Touchon*[1], *E. Rocha*[1]
[1]*Institut Pasteur, Genomique Evolutive des Microbes, Paris, France*

Backgrounds

Microbes display a remarkable interaction with the environment, which relays on the secretion of a wide variety of proteins. These effectors, although costly, are essential for cooperation, foraging, shelter, microbial warfare, and virulence. With such variety of roles, one wonders whether the different environmental factors affect similarly the evolution of the secreted proteins.

Objectives

To evaluate this question, we have studied the effect of the habitat structure and the community composition in the evolution of the bacterial secretome, with special emphasis on proteins involved in antimicrobial activity and nutrient scavenging.

Methods

Using a comparative genomics approach, we have assessed the frequency and diversity of secreted proteins associated to each environment in more than 6000 publicly available metagenomic datasets. We have evaluated with general linear mixed models the relationships between environmental factors and the secretome, paying special attention to the habitat structure and community diversity.

Conclusions

We have found that highly unstructured habitats have selected proteins minimizing their diffusibility. This maximizes the benefit from the producer while limiting the access to non producers. This association is especially important for secreted proteins involved in foraging, as they tend to be, not only less diffusible in unstructured habitats, but also less abundant in environments with a great diversity. Bacteria living in such environments also tend to carry a higher abundance of antimicrobial tools, to clash against a potentially higher diversity of contestants.

FEMS7-1000
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

AN EXPLORATION OF THE VIROME IN PATIENTS WITH ORAL LEUKOPLAKIA, PROLIFERATIVE VERRUCOUS LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA, AND ITS INTERACTION WITH THE MICROBIOTA

R. García-López[1], V. Pérez-Brocal[1], J.V. Bagan[2], A. Moya[3]
[1]Joint Unit of Research in Genomics and Health: Fundación para el Fomento de la Investigación Sanit aria y Biomédica de la Comunitat Valenciana FISABIO & Cavanilles Institute for Biodiversity and Evol utionary Biology University of València, Genomica y Salud, Valencia, Spain
[2]Universitat de València, Oral Medicine, Valencia, Spain
[3]Joint Unit of Research in Genomics and Health: Fundación para el Fomento de la Investigación Sanit aria y Biomédica de la Comunitat Valenciana FISABIO & Cavanilles Institute for Biodiversity and Evol utionary Biology University of València, Genetics, Valencia, Spain

Backgrounds

Proliferative verrucous leukoplakia (PVL) is a high-risk form of oral leukoplakia (OL), an oral lesion manifested as white hyperkeratotic plaques or warts in the human oral cavity, mainly detected in elderly women. Most PVL lesions derive in aggressive oral carcinomas, mainly oral squamous cell carcinomas (OSCC). Associations to alcohol and tobacco consumption have been discarded and, although no etiology has been confirmed for PVL, its multi-focal pattern and its high rate of recurrence hint on the involvement of an infectious agent, possibly of viral origin.

Objectives

In order to detect a potentially novel infectious agent, we studied the broad collection of viral DNA and RNA from viral communities, or virome, as well as the 16S profiles of the bacterial communities in patients with PVL, OL and OSCC.

Methods

Forty biopsies were used for the study, 10 from groups PVL, OL, OSCC, and Control. Both DNA and RNA were extracted independently for high-throughput sequencing, and the amplification of the 16S rRNA gene was carried out for bacterial composition assessments. The resulting sequences were quality-processed, filtered, assembled, identified and analyzed for taxonomic and functional profiling, depending on the set. Accessory sequences belonging to Fungi were also included in the cross-analysis of the interactions between the microbiome and the virome.

Conclusions

The detection of potential etiological agents in this thriving community is hindered by the interactions between its multiple relevant players but the virome still plays a relevant role in this bacteria-rich environment as it appears to contribute in modulating populations of preyed bacteria.

FEMS7-2202
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ASSESSMENT THE MICROBIAL COMMUNITIES ASSOCIATED TO MANILA CLAM BY MEANS OF 16S RRNA AMPLICONS

D. Gerpe[1], A. Lasa[1], S. Balboa[1], J. L.Romalde[1]
[1]CIBUS- Facultad de Biología. Universidade de Santiago de Compostela, Microbiology and Parisitology, Santiago de Compostela, Spain

Backgrounds

Clam culture represents an important economic activity in Galicia (NW Spain). All the existing studies analysed the bacterial communities using culture dependent methods, leading to an underestimation of the diversity. The introduction of the Next Generation Sequencing technologies opened a new era in the understanding the complexity of bacterial communities.

Objectives

To study the bacterial microbiota of Manila clam (*Ruditapes philippinarum*) through 16S rRNA amplicons analysis.

Methods

Samplings of the clams were carried out in summer and winter from two localities in the Galician coast. Different organs, including mantle, gills, gonads and hepatopancreas, were analysed separately. Gradient centrifugation step was performed to separate prokaryotic and eukaryotic cells. A commercial kit was employed for isolation of bacterial genomic DNA. V3/V4 region of 16S rRNA gene was amplified and sequenced at Sistemas Genómicos (Valencia, Spain) using Illumina paired-end sequencing technology.

Conclusions

In most samples analysed the main phylum was *Proteobacteria* except in mantle, gills and hepatopancreas in one of the locations where *Bacteroidetes*, Clamydiae or Tenericutes acquired more relevance. Moreover, *Firmicutes* and *Bacteroidetes* appeared in winter as the major phyla, while in summer were only detected as sporadic and very low abundant groups. Some genera as *Mycoplasma* and *Methylobacterium* appeared in high numbers in hepatopancreas and gonad respectively regardless the season. Uncultured bacteria belonging to the *Cythophagaceae* family was the major group in the mantle microbiota of clams from Redondela all over the year. *Vibrio* and *Pseudomonas* genera appeared in low representation when comparing with other studies based on culture dependent methods.

FEMS7-3129
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INFERENCE OF ECOLOGICAL INTERACTIONS IN MICROBIAL COMMUNITIES BY POPULATION TIME SERIES

C.M. Ghim[1]
[1]*Ulsan National Institute of Science and Technology UNIST, School of Life Sciences, Ulsan, Republic of Korea*

Backgrounds

With a view to controlling complex systems, the knowledge of interaction patterns among the constituent parts is essential. A recent surge of interest in microbiome research has led to massive efforts to identify microorganisms and their interactions in environmental settings, including human body. However, despite the promise metagenomic identification of microorganisms offers, reliable reconstruction of ecological interactions among those microbes is still lacking.

Objectives

We try to build a reliable mathematical inference platform to reconstruct ecological interaction networks from microbial time series, which will mark the onset of further analysis and prediction on the microbial population dynamics.

Methods

To this end, we use both empirical as well as synthetic population time series data with generalized Lotka-Volterra model. We quantitatively investigate the impact of both the quantity (duration) and quality (resolution) of the time series and evaluate the performance of various inference methods based on the accuracy and precision.

Conclusions

We evaluate the validity of the linear regression with regularization, direct integral method, and sparse linear regression for inferring interactions between microbes at the genus or higher level, and discuss applicability and limitations of each method.

FEMS7-3220
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DETERMINATION AND DIVERSITY OF SMALL FILTERABLE MICROBIAL SPECIES IN FRESHWATER

L.A. Ghuneim[1], O. Golyshina[2], D. Jones[1], P. Golyshin[2]
[1]Bangor University, School of Environment- Natural Resources- and Geography, Bangor, United Kingdom
[2]Bangor University, School of Biological Sciences, Bangor, United Kingdom

Backgrounds

Small filterable microbial species (i.e. prokaryotes with size dimensions in the 'nano' range, of 50-400 nm which have the ability to physically pass filters with pore sizes 0.2-0.45 µm) are more common than originally thought. They are known by various other names such as: 'ultra-small', 'ultramicro', 'very small', 'small cells', 'nanooorgansims', and 'nanobes'. Their role in nutrient cycling and their identity remains largely unknown due to the inability to successfully isolate in vivo.

Objectives

The goal of this research is to determine the identity and function of these small cells, and how they interact with other community members.

Methods

Using the Conwy catchment as an exemplar, we were able to assess the microbial communities in both filtered (passed through a 0.22 µm filter) and unfiltered fractions of freshwater obtained from the Conwy River. Bacterial 16S rRNA clonal library (amplicon) was generated and de novo sequencing was used to establish a basic community profile.

Conclusions

The phylogenies generated from both amplicon and de novo sequencing show evidence of 'filterable' microbial species. Additionally the community profile showed there was incredible amount of diversity within the Conwy River. The existence of the possible small filterable cells coupled with high biodiversity is indicative of a dynamic community that garners further research in terms of species identification and their contribution to overall nutrient cycling.

FEMS7-2175
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

"CANDIDATUS TREMBLAYA PHENACOLA" PPER: A BETA-GAMMAPROTEOBACTERIUM CHIMERA

R. Gil[1], C. Vargas[1], S. López-Madrigal[2], D. Santos-García[3], A. Latorre[1], A. Moya[1]
[1]Institute for Integrative Systems Biology I2SysBio- Universitat de València, Evolutionary Genetics, Paterna Valencia, Spain
[2]INSA-Lyon- Université de Lyon, Biologie Fonctionnelle Insectes et Interactions, Villeurbanne, France
[3]The Robert H Smith Faculty of Agriculture- Food and Environment- The Hebrew University of Jerusalem, Department of Entomology, Rehovot, Israel

Backgrounds

Many insects rely on bacterial endosymbionts to obtain nutrients that are scarce in their highly specialized diets. The most surprising known endosymbiotic system belongs to pseudococcinae mealybugs, where the betaproteobacterium "*Candidatus* Tremblaya princeps" and a gammaproteobacterium maintain a nested endosymbiotic consortium. In the sister subfamily Phenacoccinae, a single beta-endosymbiont, "*Candidatus* Tremblaya phenacola", has been described. We recently detected the presence of a gammaproteobacterial *trp*B gene in "*Ca.* Tremblaya phenacola" from two *Phenacoccus* species, apparently representing one of the first recorded cases of horizontal gene transfer (HGT) in bacterial endosymbionts.

Objectives

In order to identify the extent of the putative HGT, and to better understand the evolutionary history of mealybugs' endosymbiotic systems, we sequenced the genome of "*Ca.* Tremblaya phenacola" PPER from *Phenacoccus peruvianus*.

Methods

Total DNA enriched in bacterial endosymbionts was paired-end sequenced with MiSeq, and assembled using SPAdes. Unions between contigs were confirmed by PCR. Annotation was done with Prokka plus manual curation. We performed a functional analysis by comparison with other *Tremblaya* genomes and using EcoCyc and KEGG databases. The CDSs taxonomic affiliation was determined with MEGAN and phylomizer. OrthoMCL was used to generate clusters of orthologous genes. Phylogenomic reconstruction were built with PhyloPhlAn.

Conclusions

This chimeric endosymbiont presents the same biosynthetic capabilities than other mealybug endosymbiotic consortia. While the complete set of rRNA genes of this strain place it as a betaproteobacterium of the *Tremblaya* clade, nearly half of its CDSs are taxonomically affiliated to Gammaproteobacteria, forming an unprecedented evolutionary collage. A nightmare for molecular taxonomists.

FEMS7-2208
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

NATURAL OCCURRENCE OF SECONDARY BACTERIAL SYMBIONTS IN APHIDS FROM TUNISIA

R. Gil[1], S. Zouari[2], M. Reyes-Prieto[1], A. Salas Martí[1], P. Monfort Lanzas[1], A. Latorre[1], M. Ben Halima-Kamel[2]
[1]Institute for Integrative Systems Biology I2SysBio- Universitat de València, Evolutionary Genetics, Paterna Valencia, Spain
[2]Institut Supérieur Agronomique ISA- Université de Sousse Tunisie,
Cultures maraîchères conventionnelles et biologiques, Chott Mariem, Tunisia

Backgrounds

Aphids commonly face the attacks of numerous natural enemies, most notably Hymenopteran parasitoids, which are often used as biological control agents.

Many aphids harbor facultative symbionts, some of which confer protection against natural enemies. *Hamiltonella defensa* is known to provide resistance towards specific parasitoids. The degree of protection is greatly influenced by the presence of the lysogenic bacteriophage APSE. *Serratia symbiotica* and *Regiella insecticola* have also been involved in improving resistance to parasitoid wasps, albeit to a lesser extent.

Objectives

We intend to characterize different aphid species and populations from Tunisia for the presence of protective symbionts against *Aphidius transcaspicus*, a braconid parasitoid that has been selected as a biological control agent against the mealy plum aphid *Hyalopterus pruni*.

Methods

Aphids were field collected from natural populations in different locations of Tunisia. Total DNA was used for PCR amplification of symbiont 16S-23S RNA operon sequences and APSE. Amplicons were ABI-sequenced, and analyzed by BLAST to identify the bacterial species. Phylogenetic analyses were performed using Bayesian Inference with MrBayes v3.2.5 software.

Conclusions

Most analyzed populations present *Arsenophonus* sp., a widespread symbiont whose effects on aphid phenotypes are unknown. *S. symbiotica* and *H. defensa* have also been identified in several populations. All *H. defensa* strains detected possess the phage APSE-2, which has been associated with moderate protection against braconids in pea aphids.

FEMS7-0951
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EVIDENCE OF INTRASPECIFIC VARIATION IN N-ACYL L-HOMOSERINE LACTONES (AHLS) PRODUCTION BY ENVIRONMENTAL ISOLATES OF V. MEDITERRANEI

L. Girard[1], R. Lami[1], F. Vouvé[2], F. Lantoine[3], M. Suzuki[1], J. Baudart[1]
[1]Sorbonne Universités- Université Pierre et Marie Curie Paris 06- CNRS- Observatoire Oceanologique, Laboratoire de Biodiversité et Biotechnologies Microbiennes - USR 3579, Banyuls sur mer, France
[2]Université de Perpignan Via Domitia, Laboratoire B.A.E, Perpignan, France
[3]Sorbonne Universités- Université Pierre et Marie Curie Paris 06- CNRS- Observatoire Oceanologique, Laboratoire d'Ecogéochimie des Environnements Benthiques - UMR 8222, Banyuls sur mer, France

Backgrounds

Quorum sensing (QS) is a mechanism enabling bacteria to coordinate their genetic expression, behavior and physiological responses. A large panel of molecules named autoinducers (AI) are involved in these regulations, including AHLs (N-acyl L-homoserine Lactones). Many studies have revealed intraspecific variability of AHLs among members of the same *Vibrio* species (e.g. *V. fischeri*, *V. anguillarum*, *V. metschnikovii*, *V. splendidus*). However, despite current knowledge on the production patterns, little is known about the ecological processes that drive AHL production phenotypes in marine environment.

Objectives

The aim of this study was to assess the temporal dynamic of AHLs producing *V. mediterranei* isolates and to evaluate the impact of environmental variables on these phenotypic variations.

Methods

We investigated the capacity of *V. mediterranei* isolates, collected in Salses-Leucate Mediterranean lagoon, to produce AHLs fortnightly during Spring/Summer 2015 and 2016. The analysis of the *V. mediterranei* diversity was investigated by combining ERIC-PCR fingerprinting and phylogenetic analysis of *gyrB* sequences. The AHLs characterization was based on a biosensor based assays, HPLC fractionation and activity-screening prior to UHPLC-HRMS/MS analysis.

Conclusions

Our results revealed a temporal variation of *V. mediterranei* isolates with a succession of different AHL production phenotypes. Remarkably we observed a ERIC-PCR genotype of *V. mediterranei* able to produce a different set of AHLs, in identical culture conditions, over time. The possible interactions between environmental variables and *V. mediterranei* AHL production phenotypes will be discussed.

FEMS7-3048
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INACTIVATION MECHANISMS OF ENTEROCOCCUS FAECALIS BY SODIUM HYPOCHLORIDE, CHLORINE DIOXIDE AND ELECTRO ACTIVATED WATER

Z. Girgin Ersoy[1], Ö. Türkay[2], S. Barışçı[2], B. Çınar[1], S. Tunca Gedik[1]
[1]Gebze Technical University, Molecular Biology and Genetics, Kocaeli, Turkey
[2]Gebze Technical University, Environmental Engineering, Kocaeli, Turkey

Backgrounds

Chlorine based disinfectants are commonly used disinfectant agents in a variety of different application areas. There are numerous studies focusing on disinfectant dose and contact time for the removal of different microorganisms by using these agents. However, their mode of action on inactivation of microorganisms has not been comprehensively studied.

Objectives

Therefore, in this study, the effectiveness of three chlorine based disinfectants sodium hypochloride (NaOCl), chlorine dioxide (ClO_2) and electro activated water (EAW) were compared and their bacterial inactivation mechanisms were deeply investigated. *Enterococcus faecalis* was selected to reveal inactivation mechanisms of these disinfectants on a Gram positive bacteria.

Methods

For this purpose, supernatant analysis (determination of cell membrane integrity, measurement of potassium (K^+) leakage and measurement of conductivity) and cellular analysis (measurement of TTC-dehydrogenase activity, determination of DNA damage, SDS-PAGE analysis, measurement of lipid peroxidation and FTIR analysis) were carried out.

Conclusions

According to our results, EAW was the most effective disinfectant by killing bacteria in a very limited time even at low concentrations. All the disinfectants tested brought about DNA, protein and ion leakage due to disruption of cellular membrane; finally caused cell death because of breaking down the ion balance between inside and outside of the cell. Additionally, disinfectant agents could pass through the membrane and effect cellular structures such as DNA, proteins and lipids of the cell negatively. In conclusion, EAW is favorable agent amongst three chlorine based disinfectants due to its strong and environmental friendly character.

FEMS7-3049
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MECHANISMS OF ESCHERICHIA COLI AND ENTEROCOCCUS FAECALIS INACTIVATION BY POTASSIUM FERRATE

Z. Girgin Ersoy[1], S. Barışçı[2], N. Genel[1], H. Öztürk[3], Ö. Türkay[2]
[1]Gebze Technical University, Molecular Biology and Genetics, Kocaeli, Turkey
[2]Gebze Technical University, Environmental Engineering, Kocaeli, Turkey
[3]Kocaeli University, Environmental Engineering, Kocaeli, Turkey

Backgrounds

Ferrate (VI) has been used as a greener oxidant in water treatment with its dual effect (i.e. oxidation and coagulation character) for years. Besides its ability for chemical oxidation of organic substances, ferrate (VI) can also be used as a disinfectant agent for the removal of microorganisms. In recent years, some of studies used potassium ferrate in this manner, but the primary focus of these studies was to evaluate the effect of contact time and disinfectant concentration.

Objectives

For this reason, the scope of this study was to reveal the bacterial inactivation mechanism of potassium ferrate on a cellular scale. Because of their different membrane structures, *Escherichia coli* and *Enterococcus faecalis* were selected as a Gram positive and a Gram negative bacteria, respectively.

Methods

The inactivation mechanism of potassium ferrate (K_2FeO_4) on each microorganism was determined by supernatant analysis (determination of cell membrane integrity and measurement of potassium (K^+) leakage and measurement of conductivity) and cellular analysis (measurement of TTC-dehydrogenase activity, determination of DNA damage, SDS-PAGE analysis, measurement of lipid peroxidation and FTIR analysis).

Conclusions

The results showed that potassium ferrate killed both Gram positive and Gram negative bacteria in a short time by working on cellular membrane structures. On one hand, the disruption of cell membrane integrity caused leakage of cellular structures (such as DNA, protein…) and ions occurred. On the other hand, potassium ferrate damaged cellular structures directly. Using many different methods, this study provides the fundamental mechanism of Ferrate (VI) on bacterial inactivation.

FEMS7-2228
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BEHAVIOR OF STREPTOMYCES SCABIES, MYXOCOCCUS XANTHUS AND RHODANOBACTER THIOOXYDANS CO-CULTIVATED ON POTATO SUBERIN

L. Giroux[1], *S. Lerat*[1], *C. Beaulieu*[1]
[1]*Université de Sherbrooke, Biologie, Sherbrooke, Canada*

Backgrounds

Suberin is a complex biopolymer found in various plant tissues such as the potato periderm. Suberin acts protective layer against pathogen. Bacteria cannot easily degrade suberin and its degradation was attributed to fungi for a long time. The common scab pathogen *Streptomyces scabies* was shown to be able to grow with suberin as a carbon source and to degrade it. A previous study demonstrated that some bacteria found in the potato soil could potentially be involved in suberin degradation. This proteomic study allowed the identification of lipases from *Myxococcus xanthus* and *Rhodanobacter thiooxydans* when a soil community was exposed to potato suberin.

Objectives

In a 60-days experiment, the capacity of *M. xanthus, R. thiooxydans* and *S. scabies* alone or in combination to degrade suberin was assessed.

Methods

The strains were grown in minimal media with suberin as the carbon source and sampling was done every ten days. Various enzymatic assays were performed to evaluate the enzyme production of the strains and TEM microscopy was performed to detect potential degradation.

Conclusions

Extracellular protein production decreases during the experiment but esterase activity of the supernatant was higher in the combination of strains. The strains survival was also assessed in the strains combination. The survival rate of *M. xanthus* was better when grown with the two other bacteria than alone and as for the *R. thiooxydans,* growth showed decline in the presence of the two other bacteria. The combination of the three strains performed better in most enzymatic assays suggesting greater capacity to degrade suberin.

FEMS7-0910
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CO-OCCURRENCE NETWORKS AND ASSEMBLY PROCESSES IN SOIL DENITRIFIER COMMUNITIES

M. Goberna[1], *S. Donat*[1], *E. Pérez-Valera*[1], *S. Hallin*[2], *M. Verdú*[1]
[1]*Centro de Investigaciones Sobre Desertificación, Ecología, Moncada, Spain*
[2]*Swedish University of Agricultural Sciences, Microbial Ecology, Uppsala, Sweden*

Backgrounds

Denitrifiers possessing *nirS* or *nirK* genes are thought to be ecologically distinct since they respond differently to soil abiotic factors. Furthermore, they could have different fitness associated with the costs of synthesizing heme- or Cu-dependant nitrate reductases. Thus, several assembly processes, ie. abiotic filtering, competition based on fitness differences or on limiting similarity, might simultaneously operate to structure the soil denitrifier communities.

Objectives

We aimed to unravel the ecological processes shaping the denitrifier communities before and after fire, which shifts resource availability and thus likely the strength of competition.

Methods

We analysed co-presence and mutual exclusion links in *nir*-based networks and quantified *nirS* and *nirK* before and during one year after an experimental fire.

Conclusions

Network analysis on *nirS* and *nirK* sequences revealed that co-presence links mostly occurred within the same *nir* variant, while exclusion links were equally distributed within and between *nir* variants. Under pre-fire conditions, co-presence *nirS-nirS* links occurred more often than expected by chance, reflecting the similar niche requirements of ecologically similar microbes. Mutual exclusion links, however, were overrepresented for *nirK-nirK* and *nirS-nirK*. While exclusion links between *nir* variants (*nirS-nirK*) can result from niche segregation, those within a single variant (*nirK-nirK*) suggest competitive exclusion based on limiting similarity that precludes the co-existence of functionally similar organisms. Fire erased the signals of competition between *nirK*-bearing microbes probably by reducing resource limitation, despite quantitative PCR revealed a significant increase in *nirK* copy numbers. Our results indicate that fire alters the relative contribution of assembly processes in shaping the communities of soil denitrifiers.

FEMS7-2317
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE RELEVANCE OF THERMOPHILES IN SOILS. AN ENZYMATIC APROACH

E. Gómez[1], *J. Delgado*[1], *J. González*[1]
[1]*IRNAS-CSIC, Biogeoquímica- ecología vegetal y microbiana, Seville, Spain*

Backgrounds

In the last years, the role of thermophilic microorganisms in soils is starting to be understood above all within an scenario of periodically changing temperature and other environmental conditions. Unexpectedly, the presence of these thermophiles has been confirmed in a wide range of environments. However, their presence and activity are scarcely understood. Extremophilic enzymes are known to be able to maintain their stability (and thus their function) under extreme conditions such as high temperature or dessication. These properties are attracting the interest on these enzymes as a source of new tools for biotechnology. In relation to the biogeochemical cycles of elements, the extracellular enzymatic activity is generally the limiting processe for the mineralization of polymers and recalcitrant organic matter by microorganisms.

Objectives

Evaluate the enzymatic activity by thermophilic microorganisms in soils in a variety of environments.

Methods

In this study, we used fluorogenic substrates to determine different enzymatic activities under different environmental and laboratory conditions.

Conclusions

Our data show that the activities of extracellular enzymes from thermophiles represent the maximum peak of activity in natural soils and sediments. These results suggest that the thermophilic enzymes could play critical roles in the precessing of organic mater in terrestrial environments. These findings contribute to the understanding of the ecology and functioning of microorganisms in soils, specially on arid and semi-arid environments. This is highly relevant in the current climate change framework when a rise of extreme high temperature and dessication events are expected.

FEMS7-1227
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CAROTENOID – PRODUCING BACTERIA – A CAUSE OF MURAL PAINTING BIODETERIORATION IN SOME ROMANIAN HISTORICAL MONASTERIES

I. Gomoiu[1], M.I. Enache[2], E.S. Neagu[2], I.M. Cortea[3], R. Radvan[3], L.R. Cojoc[2]
[1]National University of Arts Bucharest, Conservation and restauration, Bucharest, Romania
[2]Institute of Biology Bucharest of the Romanian Academy, Microbiology, Bucharest, Romania
[3]National Institute for Research and Development in Optoelectronics,
Center of Excellence for Restoration by Optoelectronic Techniques – CERTO, Magurele-Ilfov, Romania

Backgrounds

Carotenoids are widely distributed between extremophiles. Some halophilic bacteria produce carotenoids as adaptation to salt stress and, in the case of mural painting, their accumulation results in the modification of the original color, producing pink or red pigmentation.

Microclimate peculiarities of Hurezi and Humor monasteries and the presence of efflorescences offer favorable conditions for halophilic bacterial colonization and biodeterioration.

In the refectory of Hurezi monastery, pink pigmentation is visible on all walls and in Humor Monastery, it was identified up to the height of 0,5m in the pre-nave and up to 0,7m in the chamber tombs.

Objectives

The aim of present work is to highlight a new corroboration of scientific methods to identify carotenoid-producing halophilic bacterial strains involved in biodeterioration of mural paintings.

Methods

The pink pigmentation observed by naked eye was further analyzed by optical and scanning electron microscopy. Microbiological and molecular techniques were used for isolation and identification of carotenoid-producing bacteria. Carotenoid pigments were identified by FTIR (Fourier transform infrared spectroscopy) on samples of mortar, pictorial layer and on pure bacterial cultures.

Conclusions

The correlation of the laboratory data with those registered on the mural painting allowed the identification and attribution of pink biopigmentation to *Halobacillus naozhouensis.* The biopigmentation was put in evidence on most analyzed samples: mural painting, repaintings, infilling mortars clearly stating the presence of carotenoid pigments identified in the case of this halophilic bacterial strain.

FEMS7-0993
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

STUDY OF POTENTIAL OF OIL DEGRADATION BY MICROORGANISMS ISOLATED FROM THE CASPIAN SEA REGION

A. Goncharova[1], T. Karpenyuk[1], Y. Tufuminova[1], A. Kalbayeva[1], S. Yergaliyeva[1], A. Platayeva[1], M. Zavorotnaya[1]
[1]al-Farabi Kazakh National university, Research Institute of Ecology problems, Almaty, Kazakhstan

Backgrounds

The basis of biotechnological purification of the environment from oil and petroleum products are biological products containing viable cells of hydrocarbon oxidizing microorganisms. The problem of creating effective and accessible microbial consortia to eliminate dangerous oil pollution is very relevant.

Objectives

We have studied the growth activity and biodegradation of petroleum hydrocarbons of local deposits by microorganisms-destructors allocated in the Caspian region.

Methods

Methods: fluorimetric method for determining of oil concentration, emulsification index was determined by Rosenberg method, BATH-test to determine the hydrophobicity of the cell wall, gravimetric and nephelometric methods for determining the growth of microorganisms on the 1 and 3% oil.

Conclusions

Based on the active growth of microorganisms on solid and fluid medium containing 1% oil (as a sole carbon source) were selected 57 cultures, which were measured for biomass growth, cell wall hydrophobicity, emulsification index and the percentage of oil degradation. We have selected cultures for which the percentage of oil degradation ranged from 91,1 ± 4,5; to 49,7 ± 3,5, emulsification index values ranged from 52.7% to 43.9%, the hydrophobicity growth varied from 46.1% to 6.7%, biomass was increased (in 10 days) from 200% and 3000%. Selected cultures after identification were assigned to the following genera: *Achromobacter, Ochrobactrum*, *Stenotrophomonas, Roseomonas, Rhodococcus, Sphingobacterim.* These organisms have a high potential of hydrocarbons degradation, comparable to the activity of the commercial product BK-Oil Buster, they are not antagonists to each other and can be used to create on their basis the consortiums for their further use in bioremediation.

FEMS7-1039
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

IMPACT OF HOMOLOGOUS RECOMBINATION ON THE EVOLUTION PROKARYOTIC CORE GENOMES

P. Gonzalez-torres[1], F. Rodriguez-mateos[2], J. Anton Botella[3], T. Gabaldon[1]
[1]Centre for Genomic Regulation CRG, Bioinformatics and Genomics Program, Barcelona, Spain
[2]University of Alicante, Matemática Aplicada, Alicante, Spain
[3]University of Alicante, Physiology- Genetics- and Microbiology, Alicante, Spain

Backgrounds

Metagenomic and genomic studies carried out in recent years suggest that intraspecific homologous recombination (HR) could play an important role in the microevolution of many microorganisms, with this mechanism being sometimes the major evolutionary force. However, such studies are still scarce, focused only on few aspects or life style strategies and heterogeneous in terms of distribution over the prokaryote phylogeny.

Objectives

To assess whether HR may act as a prevalent mechanism in the evolution of core genomes, explore which factors determine its incidence and finally clarify HR effect on the microdiversity, adaptative events and generation of cohesive population structures within bacterial specie

Methods

Here we deal with this open question using an extensive study that involves 338 strains of 54 prokaryotic species covering a wide distribution of phylogenetic groups and life-styles. In order to elucidate the most relevant factors that determine the impact and distribution of HR, genomic and ecological elements that favour or hinder the horizontal transfer as restriction modification systems (RM), CRISPR-Cas systems or competence genes (com) were considered.

Conclusions

Life-style and ecology were the factors most associated with the distribution of HR events. HR exchange gene patterns showed a clear functional structuring according to different ecological strategies, adaptation processes, environmental pressure and fitness. In addition, competent species and those with great presence of MR systems exchanged greater events than the rest. HR contributes to the homogenization of core genomes and maintains cohesive population structures thus affecting significantly the microevolution of prokaryotic species.

FEMS7-2236
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

STUDY OF AIRBORNE BACTERIOPHAGES AND ANTIBIOTIC RESISTANCE GENES IN TENERIFE, CANARY ISLANDS, SPAIN

C. Gonzalez-Martin[1], N.M. Coronado-Alvarez[2], N. Teigell-Perez[1], R. Diaz-Solano[1], F.J. Exposito[3], J.P. Diaz[3], D.W. Griffin[4], B. Valladares[1]
[1]Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias, Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias, San Cristobal de La Laguna, Spain
[2]Hospital Universitario San Cecilio, Antimicrobial Research Laboratory. Infectious Diseases Department, Granada, Spain
[3]Grupo de Observación de la Tierra y la Atmósfera GOTA, Universidad de La Laguna, San Cristóbal de La Laguna, Spain
[4]St. Petersburg Coastal and Marine Science Center, United States Geological Survey, Florida, USA

Backgrounds

Dispersion of antibiotic resistance genes by bacteriophages is an important agronomic and public health issue. This type of viruses may transform innocuous bacteria into pathogens or modify their resistances to antibiotic-based treatments. Recent studies have identified the presence of bacteriophages in air samples collected from different environments, implicating the atmosphere as a limited, but important, source for the dissemination of antibiotic resistance genes.

Objectives

1. To detect, identify and analyze the presence of bacteriophages and associated antibiotic resistance genes in the atmosphere.
2. Analyze the relationships between the microbiological results and different clim atic variables.

Methods

Atmospheric samples were collected in an urban area of Tenerife, Canary Islands, Spain. After concentration and nucleic acid extraction, samples were screened for bacteriophage genomes (DNA and RNA phages) and antibiotic resistance genes. Climatic variables, such as the occurrence of African dust storms, seasonality, origin of the air mass and particle matter levels were analyzed and compared with the microbiology data to detect possible correlations.

Conclusions

Positive results were obtained for F – DNA bacteriophages (10%) and antibiotic resistance gene TEM (6.2%). No statistical relationships were observed between the microbiological data and the occurrence of African dust storms, the origin of the air masses or the PM values. F – DNA phages detection was significantly correlated with cooler seasons. This is the first study describing the presence of these viruses and antibiotic resistance genes in an outdoor urban environment in the Canary Islands, Spain.

FEMS7-2824
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

AIRBORNE VIABLE BACTERIAL COMMUNITY IN AN URBAN AREA IN TENERIFE, CANARY ISLANDS, SPAIN.

N. Teigell Perez[1], C. Gonzalez-Martin[1], R. Diaz Solano[1], F.J. Exposito[2], J.P. Diaz[2], D.W. Griffin[3], B. Valladares[1]
[1]Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias,
Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias,
San Cristobal de La Laguna, Spain
[2]Grupo de Observación de la Tierra y la Atmósfera GOTA, Universidad de La Laguna,
San Cristobal de La Laguna, Spain
[3]USGS, St. Petersburg Coastal and Marine Science Center, Florida, USA

Backgrounds

Despite their potential implications for human health, agriculture and other ecosystems, and their ability to survive long-range transport, information about airborne microorganisms is scarce. Dust storms are considered a long-range mechanism of dispersion for microorganisms. Due to the proximity of the Canary Islands to the African continent, the archipelago is commonly affected by Saharan dust intrusions.

Objectives

1. To study the viable airborne bacteria community in the atmosphere of an urban area in Tenerife.
2. Analyze the relationship between the microbiological results with climatic variables.

Methods

Atmospheric samples were collected in an urban area of Tenerife. Isolation and identification of viable bacteria was performed using culture followed by 16 rRNA PCR and sequencing. Climatic variables, such as the influence of African dust storms, seasonality, origin of the air mass and meteorological parameters (rain, temperature, humidity) were analyzed to determine possible correlations.

Conclusions

Fifty-three samples were collected and a total of 732 bacteria were isolated. *Bacillus* sp. (21.72%), *Arthrobacter* sp. (17.49%) and *Staphylococcus* sp. (11.48%) were the most common among the fifty-eight genera identified. Potential pathogens for animals and plants were detected. No significant correlation was noticed between climactic variables and microorganisms. However, isolates within Firmicutes and Actinobacteria phyla were more abundant during African dust days. Also, Gram-positive bacteria were predominant over Gram-negative. Gram-positive bacteria increased during African dust days while Gram-negative increased during non-dust days. This is the first study in the Canary Islands regarding the composition of airborne bacterial communities and their relationship with different environmental factors.

FEMS7-1732
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

FUNGAL ENDOPHYTES ISOLATED FROM ARID PLANTS OF ANDALUCIA: PRODUCTION OF NEW ANTITUMOR AND ANTIFUNGAL ACTIVITIES BY THE ADDITION OF ADSORPTIVE POLYMERIC RESINS.

V. Gonzalez-Menendez[1], G. Crespo[2], N. de Pedro[3], C. Diaz[3], J. Martin[2], C. Toro[1], F. Muñoz[2], C. Justicia[2], F. Vicente[3], F. Reyes[2], J.R. Tormo[2], O. Genilloud[4]
[1]Fundacion MEDINA, Microbiology, Granada, Spain
[2]Fundacion MEDINA, Chemistry, Granada, Spain
[3]Fundacion MEDINA, Screening, Granada, Spain
[4]Fundacion MEDINA, Scientist Director, Granada, Spain

Backgrounds

Arid zones in Andalucía have native plant communities possessing distinctive characteristics to survive in special conditions that have led to the existence of a large number of poorly studied endemic plants. It is precisely this singularity which turns them into a potential source for the discovery of novel fungal symbionts, as well as host-specific endophytes not described yet. Addition of adsorptive polymeric resins in fungal fermentations has been described to promote the production of new secondary metabolites as a tool to consistently generate new compounds with potential biological activities.

Objectives

To evaluate the effects of addition of resins on our collection of fungal endophyte fermentations for the generation of new extracts with antitumor and antifungal activities.

Methods

A total of 346 fungal strains isolated from 54 selected plant species from these ecosystems were characterized morphologically as well as on the basis of their ITS/28S ribosomal gene sequences. The strains were grown in four different media in the presence and absence of selected resins according to our previous studies. Fermentation extracts were evaluated for the presence of induced cytotoxic activities against HepG2 cell line and antifungal activities against the human fungal pathogens *Aspergillus fumigatus* and *Candida albicans*.

Conclusions

Twelve compounds were purified by chromatographic techniques and identified as being the active secondary metabolites induced in the fermentations by the adsorptive polymeric resins. Our results also confirmed the biodiversity richness of the plants of Andalusian deserts as an untapped source of new host-specific fungal strains with the potential to produce new bioactive compounds.

FEMS7-2895
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ARSENIC RESISTANCE OF PLANT-MIOCROBES ASSOCIATION AS A TOOL TO RESTORED CONTAMINATED ENVIRONMENTS

N. Gonzalez[1], M. Diaz[1], R. Simarro[1], S. Garcia[2], M.A. Quijano[2], L.F. Bautista[3], J.F. White Jr[4], J. Lopez[1], M.D.C. Molina[1]
[1]Universidad Rey Juan Carlos, Department of Biology and Geology- Physics and Chemistry, Móstoles, Spain
[2]Universidad Politecnica de Madrid,
Departmento de Ingenieria Civil- Hidraulica y Ordenacion del territorio, Madrid, Spain
[3]Universidad Rey Juan Carlos, Department of Chemical and Environmental Engineering, Móstoles, Spain
[4]Rutgers- The State University of New Jersey, Department of Plant Biology, New Brunswick, USA

Backgrounds

Arsenic (As) is a highly toxic metalloid that accumulates in the environment as a result of natural and anthropogenic processes. The major form of As in aerobic soils is As(V), which is structurally a chemical analogue of phosphate and enters plant root tissue via phosphate transporters. Plants and microorganisms can play a vital role in arsenic species transformation. Reduction of As (V) to As (III) and biomethylation to mono or dimethyl arsenic, trimethylarsine oxide and volatile trimethylarsine, through the oxidation of organic matter or the utilization of As (V) as an electron acceptor, have been reported as detoxification mechanism. In addition, As (III) may form complexes with thiol compounds such as phytochelatins (PCs) and glutathione (GSH), in order to convert inorganic As into less toxic organic forms. The association of plant-microbiome (rhizosphere and endosphere) and their interactions have been recently considered as a Metaorganism with important ecological, phytotechnological and healthy environmental implications.

Objectives

The aim of this study was to identify the rizhosphere and endosphere microbial composition in a Mediterranean plant such as *Jasione montana* and extimate the minimal lethal As concentration of bacteria and fungi endophytes. We selected this plant for being able to live in highly contaminated soils with arsenic and behave like a resistant plant.

Methods

Jasione montana is a Mediterranean native terrestrial plant collected in the vicinity of the Mónica mine (NW Madrid, Spain), from As soil polluted (from 0.3 to 30 g kg^{-1}) by past mining activities. Bacterial rizosphere DNA from *J. montana* rizhosphere was extracted for metabarcoding sequencing Illumina. Bacteria and fungi from *J. montana* endosphere were isolated and exposed to an increasing gradient of As (V) to estimate the minimal lethal As(V) concentration of the microbial species. Arsenic speciation analysis was performed by high performance liquid chromatography-photo-oxidation-hydride generation-atomic fluorescence spectrometry (HPLC-(UV)-HG-AFS), to evaluate arsenic species transformation. Total arsenic concentration was determined by ICP-AES to detect the presence of unidentified arsenic species.

Conclusions

J. montana rizosphere showed a similar bacterial composition at all As concentration levels in soils, with 30 % of Actinobacteria, 20% of Proteobacteria and 10% of Acidobacteria. Endophytic bacteria as

Pantoea sp, *Kocuria rosea*, *Pseudarthrobacter oxydans, Arthrobacter siccitoleran*, and fungi as *Fusarium* sp, *Fusarium oxysporum*, *Umbelopsis isabellina*, *Mortierella lignicola* and *Curvuraria protuberata* strain were identified in *J. montana* with a minimal lethal As concentration of around 250 mM for bacteria and 50 mM for fungi.

Regarding arsenic speciation studies, reduction of As (V) to As(III) was detected, but not methylated species. However, between 20-40% of arsenic remained as unidentified species, probably due to their biotransformation to thiol compounds complexes, which cannot be detected with the analytical method applied.

FEMS7-0810
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE HIDDEN DYNAMICS OF THE RARE MICROBIAL BIOSPHERE IN COASTAL WATERS OF EASTERN AUSTRALIA

K.M.E. Goossen[1], J.P. Bowman[2], M.V. Brown[3], J. van de Kamp[1], L. Bodrossy[1]
[1]CSIRO Oceans and Atmosphere, Environmental Genomics, Hobart, Australia
[2]University of Tasmania, TIA - Research Institute, Hobart, Australia
[3]University of New South Wales, School of Biotechnology and Biomolecular Science, Sydney, Australia

Backgrounds

The roles rare microorganisms play in maintaining biodiversity and modulating biogeochemical processes such as carbon and nutrient cycling are not well understood.

Objectives

To address this, we analysed Illumina (Mi/Seq) based bacterial and archaeal 16S rRNA gene amplicon sequencing data from monthly time series.

Methods

Samples were collected from six depths at each of three coastal National Reference Stations (NRS) operated in conjunction with the Integrated Marine Observing System (IMOS). These stations are located in distinct bio-regions, ranging from sub-tropical to cool temperate, along Australia's eastern seaboard. Rare and abundant taxa were defined as having abundances below and above 0.1% of total reads of all samples per station, respectively.

Conclusions

The rare microbial taxa comprised approximately 98% of the total operational taxonomic units (OTUs) observed and 34% were shared amongst all three stations. At two stations the rare taxa increased in abundance in deeper waters compared with the surface samples. Rare and abundant taxa were classified as Persistent (>75%), Intermittent (25-75%) and Transient (<25%) based on their occurrence across all samples. During late winter one station showed a decrease of the abundant persistent OTUs over two years at ~ 30m depth, with a concomitant increase in the rare transient OTUs. Based on these criteria the rare biosphere displayed different patterns to the abundant taxa in relation to depth, season and latitude. We observed that rare taxa can increase in abundance when environmental conditions are advantageous sometimes replacing nominally abundant taxa, suggesting an important role in maintaining ecosystem functionality under fluctuating conditions.

FEMS7-1191
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DETECTION OF BACTERIA ASSOCIATED WITH PM10 PARTICULATE MATTER IN MEDELLIN – COLOMBIA

D. Grajales[1], C. Zapata[2], O. Montoya[1], C. Moreno[1]
[1]Universidad Nacional de Colombia - Sede Medellín, Biociencias, Medellín, Colombia
[2]Universidad Nacional de Colombia - Sede Medellín, Geociencias y Medioambiente, Medellín, Colombia

Backgrounds

In recent years, particulate matter air pollution has increased in major cities worldwide and its effects have been directly related to health conditions.

Objectives

The aim of this research was to evaluate the presence of bacteria associated with particulate matter with aerodynamic diameter less than 10 micrometres and its correlation with meteorological variables.

Methods

In this study, a Hi-Vol air sampler was implemented to collect the PM10 particulate matter in two seasons of the year (Wet and Dry) at the MED-UNFM station of the Universidad Nacional de Colombia (Medellín, Colombia, South America). Environmental parameters such as concentration, temperature, humidity and wind speed were determined and the bacterial diversity associated with PM10 filters was evaluated using culture-dependent and molecular identification methods. Additionally, hemolytic activity was evaluated in the isolates identified

Conclusions

Molecular characterization analyses revealed the presence of predominant bacteria in both seasons, belonging to the phylum Firmicutes and predominant bacteria from the phyla Proteobacteria and Actinobacteria in dry seasons with species such as *Bacillus megaterium*, *B. pumilus*, *Escherichia hermannii*, *Arthrobacter gandavensis* and *Staphylococcus aerlettae*. Some of these species have been considered as possible human pathogens with possible antibiotic resistance. Sixty-two percent (62%) of the isolates had beta-haemolytic activity. Temperature and humidity increased the amount of bacteria captured in the PM10 filters. These results provide important information to understand the distribution of bacteria associated with different concentrations of particulate material in the air and are of interest because of their influence on the environmental health and air quality of the region.

FEMS7-1081
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MULTIDRUG-RESISTANT ESCHERICHIA COLI AND OTHER ENTEROBACTERIACEAE SPECIES DETECTED IN MARINE BIVALVES HARVESTED ALONG THE NORWEGIAN COAST

D. Grevskott[1], C. Smith Svanevik[1], M. Sunde[2], A.L. Wester[3], B.T. Lunestad[1]
[1]National Institute of Nutrition and Seafood Research, Contaminants and Biohazards, Bergen, Norway
[2]Norwegian Veterinary Institute, Bacteriology- Fish and Animal, Oslo, Norway
[3]Norwegian Institute of Public Health, Bacteriology, Oslo, Norway

Backgrounds

The mechanisms for development of resistance in bacteria residing in the coastal environment are far from elucidated. Bivalve molluscs filtrate large amounts of seawater daily, and retain smaller particles, including free and particle-bound bacteria. These bacteria may originate from humans and livestock animals either via sewage, by runoff from land, or representatives of the wild fauna, such as birds or marine mammals. Bivalves are therefore excellent indicators for faecal contamination by reflecting the load of *Escherichia coli* and other Enterobacteriaceae species present in the water column at a given location.

Objectives

The objective of this study was to examine for antibacterial resistance among *Escherichia coli* and other species in the Enterobacteriaceae family isolated from bivalve molluscs.

Methods

A total of 549 bivalve samples were examined applying the standardised Most Probable Number (MPN) EU-method (ISO-16643-3). The disc diffusion method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were performed with 26 agents on all 199 isolates recovered. A selection of ten multidrug-resistant *E. coli* isolates were subjected to Whole-genome Sequencing (WGS). Multiple-locus Variable Number Tandem Repeats Analysis (MLVA) was applied on a selection of 30 resistant *E. coli* isolates in order to compare isolates from bivalves with community-acquired strains causing bacteraemia in humans.

Conclusions

Totally 75 (38%) of the isolates showed antibacterial resistance, and 9 (5%) were found to be multidrug-resistant, including resistance towards third-generation cephalosporins. This study demonstrates that bivalves represent an important tool for monitoring of antibiotic resistant *E. coli* and other Enterobacteriaceae species in the coastal environment.

FEMS7-2686
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BACTERIAL BIODEGRADATION NETWORKS FOR POLYCYCLIC AROMATIC HYDROCARBONS REMOVAL IN POLLUTED SOILS

J. Vila[1], M. Tauler[1], S. Jiménez-Volkerink[1], M. Grifoll[1]
[1]University of Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain

Backgrounds

Bacterial metabolic pathways for the biodegradation of single polycyclic aromatic hydrocarbons (PAHs) by pure cultures are well established. The challenge now is to identify the actual *in situ* cooperative metabolic routes driving PAH removal in polluted environments, where these compounds are constituents of complex mixtures exposed to versatile microbial communities under diverse environmental conditions.

Objectives

In this study, we investigate the bacterial populations and processes involved in the transformation and eventual elimination of PAHs, oxy-PAHs and N-PAHs, in a creosote-polluted soil (PAH 8,500 ppm) from a historical site, amended with nutrients and incubated in aerobic conditions.

Methods

Depletion of substrates and formation of metabolites (GC-MS) is correlated with the dynamics of total and active phylogenetic groups (pyrosequencing of 16S rRNA genes and transcripts), and the abundance and expression (qPCR) of key functional genes (Ring Hydroxylating Dioxygenases, RHDs).

Conclusions

The stimulation of the natural communities resulted in a extensive removal of PAHs (96%). The initial fast removal of 2-3 ring PAHs was associated to members of *Pseudomonas* and *Pseudoxantomonas* harboring nah-like RHDs. Ketones and quinones were formed and transiently accumulated, being later degraded by alternative microbial populations. 4-Ring PAHs were removed by members of *Sphingobium,* unclassified *Gammaproteobacteria* and mycobacteria, the low relative abundance of the latter being compensated by their level of expression. Cometabolism played a key role, the resulting oxy-PAHs acting as metabolic nodes in complex metabolic networks. Analysis of RHDs was limited by the availability of primers and the knowledge of RHD diversity.

FEMS7-2764
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BIODEGRADATION OF BENZ(A)ANTHRACENE AND CHRYSENE IN PAH-POLLUTED SOILS

S. Jiménez-Volkerink[1], J. Vila[1], J.M. Nieto[1], M. Grifoll[1]
[1]University of Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain

Backgrounds

Benz(*a*)anthracene (BaA) and chrysene (CHY) are 4-ring polycyclic aromatic hydrocarbons (PAHs) of known genotoxicity and carcinogenicity. Due to their low water solubility and chemical stability, they are resistant to extensive microbial degradation, being enriched in historically polluted sites. Although a few degrading bacterial strains have been reported, little is known about the microbial populations and interactions involved in the *in situ* biodegradation of these compounds in polluted soils.

Objectives

The general objective of this work is to obtain and thoroughly characterize BaA and CHY-degrading bacterial communities from a creosote-polluted soil, isolate their components, describe the functions involved, and, finally, confirm the modeled processes in polluted soils.

Methods

In a first phase we have assessed the biodegradation and microbial community shifts in sand-in-liquid soil microcosms spiked with BaA, CHY or benz(*a*)anthracene-7,12-dione, by using chemical (GC-FID) and molecular analyses (16S rRNA-PCR-DGGE).

Conclusions

The degradation kinetics of CHY, BaA and BaA-dione in the microcosms, suggested a cometabolic attack of BaA and CHY when together. Analogous populations could degrade BaA and BaA-dione, as they presented similar biphasic plots. DNA-based analysis did not reveal substantial changes in the structure of the total bacterial community; however, the further RNA-based analysis suggested a catabolic redundancy between different active phylogenic groups. The actual relevance of these phylotypes in polluted soils will be confirmed by mining pyrosequencing data, including total and active microbiome analysis, from a previous bioremediation assay.

FEMS7-3060
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

VIROME DIVERSITY IN TROPICAL WATER BODIES AND WATER CATCHMENTS IN SINGAPORE

X. GU[1], Q.X.M. Tay[2], N. Saeidi[1], S.G. Goh[1], K. Ariel[3], J. Thompson[4], K.Y.H. Gin[1]
[1]National University of Singapore, Department of Civil and Environmental Engineering, Singapore, Singapore
[2]Singapore-MIT Alliance for Research and Technology, Singapore-MIT Alliance for Research and Technology, Singapore, Singapore
[3]Nanyang Technological University, School of Material Science and Engineering, Singapore, Singapore
[4]Massachusetts Institute of Technology, Departments of Civil and Environmental Engineering, Cambridge, USA

Backgrounds

Viral metagenomics has developed increasingly in freshwater ecosystems. However, few studies focus on the geospatial distribution in the natural water bodies and the land use impacts on viral community. It is hypothesised that land use change will impact the characteristics of tropical water catchments and further influence virome distribution pattern. Metagenomics will be used to study the virome geospatial diversity in Singapore surface waters.

Objectives

This study seeks to understand the general distribution of virome abundance and diversity in water bodies and water catchments in Singapore and its correlation with land use. Emerging zoonotic viruses will be investigated. Besides, metagenomics data will be linked with qPCR in terms of human and plant related viruses.

Methods

A total of 38 samples were collected from 7 sampling sites (10 water bodies and 9 water catchments) during Northeast monsoon (Jan15) and inter-monsoon (Apr15). 200-ml of primary concentrate was followed by polyethylene glycol and Amicon ultrafiltration. The extracted viral nucleic acids were amplified with a random amplification protocol (Bibby and Peccia 2013) and were sent to SCELSE on Illumina Hiseq 2500 platform (2*250bp). After trimming and assembly, contigs were uploaded into Metavir pipeline for taxonomy annotation. Remapping was done by Novoalign to generate RPKM table for quantifying the virome.

Conclusions

- Overall, the majority of the annotated virome belongs to bacteriophage (65.5%).
- A higher Shannon-index virome diversity in water bodies was observed than in water catchments.
- PCoA analysis showed that land use (urbanized, agriculture and less impact areas) influenced the viral geospatial distribution community.
- 41 contigs found to have the lowest common ancestor affiliation to human viral pathogens. The most abundant human-related viruses belonged to *Hepatitis E viruses* and the second most abundant viruses were *Picobirnaviruses*.
- PMMoV RPKM analysis from metagenomics demonstrating the relative abundance and the proportion of reference-mapped reads is significantly correlated with qPCR ($0.588<r<0.879$, $p<0.05$).

FEMS7-0411
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CARBENICILLIN AND PENICILLIN TRIGGER PLANT RESPONSES ASSOCIATED TO THE PRODUCTION OF REACTIVE OXYGEN SPECIES

M. Gudiño[1,2], N. Blanco-Touriñán[1], V. Arbona[3], M.Á. Blázquez[1], F. Navarro-García[2]
[1]Instituto de Biología Molecular y Celular de Plantas CSIC-UPV, Vegetal Development, Valencia, Spain
[2]Universidad Complutense de Madrid - Facultad de Farmacia, Departamento de Microbiología II, Madrid, Spain
[3]Universitat Jaume I, Departament de Ciències Agràries i del Medi Natural, Castelló, Spain

Backgrounds

Antibiotics are considered nowadays bifunctional molecules working either as quorum-sensing signaling molecules as well as bacterial killing substances. The presence of antibiotics in soils could be due to the natural production by soil microorganisms as well as the result of anthropogenic activities. However, the impact of these compounds on plant physiology has not been thoroughly investigated.

Objectives

To evaluate the effect of several beta-lactam antibiotics (ampicillin, carbenicillin and penicillin) on the growth and development of *Arabidopsis thaliana* roots.

Methods

All essays were made with *Arabidopsis thaliana* seedlings grown in culture medium supplemented or not with 100 $\mu g \cdot mL^{-1}$ of carbenicillin, penicillin or ampicillin.

Conclusions

The exposure to penicillin and carbenicillin limited root growth and triggered the local endogenous production of ROS (reactive oxygen species) in roots, which caused an increase in the number of root hairs. Furthermore, carbenicillin-treated plants showed an altered pattern of the expression of genes involved in glucosinolate biosynthesis, and a modification of the levels of indole-glucosinolates. In summary, the presence of some beta-lactam antibiotics seems to interfere with the signaling pathways that plants use to interact with soil borne microbes.

FEMS7-2538
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIOLOGICAL ANALYSIS OF HEMODIALYSIS WATER AT THE UNIVERSITY TEACHING HOSPITAL OF YAOUNDE, CAMEROON

C. Gueguim[1], A. Ragon[2], H. Gonsu Kamga[3], F. Kaze Folefack[4]
[1]University of Yaoundé I, Microbiology, Yaoundé, Cameroon
[2]Hospital of Conception, Uro-Nephrology Laboratory, Marseille, France
[3]Microbiology Laboratory of the Hospital Center and the University Hospital of Yaoundé, Microbiologie, Yaoundé, France
[4]University of Yaoundé I, Néphrology, Yaoundé, Cameroon

Backgrounds

Rigorous control of the microbiological quality of water in hemodialysis services is important because the immune system of patients with chronic renal failure is weakened.

Objectives

To determine the microbiological quality of water for hemodialysis.

Methods

Twelve water samples were collected each month at different sites of the hemodialysis circuits **A** (inlet of filters), **B** (Outlet of filters) and **C** (outlet of the RO device) between July and October 2015 to be analyzed. The bacteria were isolated after filtration of 100 ml of water at each site through nitrocellulose membrane with 0.45 µm microporosity deposited on the surface of the *Tryptone Glucose Extract Agar* and then incubated at room temperature for 7 days. Pure bacterial isolates were identified by their cultural characters and marketed biochemical galleries. The colony count was well above the required international standards (>100 CFU / ml), a percentage of 83.3% (10/12) of non-compliance. Among the bacteria identified, nine (09) were Gram-negative bacilli including *Pasteurella haemolytica*, *Pseudomonas fluorescens, Pseudomonas paucimobilis, Aeromonas salmonicida* and *Klebsiella pneumoniae subsp ozaenae,* three (03) Gram-positive bacilli all *Bacillus sp* and six (06) Gram-positive cocci all of coagulase-negative staphylococci. The most frequently isolated bacterial genera were *Pseudomonas* (30.4%), *Staphylococcus* (26.1%), *Aeromonas* (13%), *Bacillus* (13%), *Klebsiella* (13%) and *Pasteurella* (4.3%).

Conclusions

In this study, the detection of a variety of bacteria in the hemodialysis water indicates the need for regular monitoring of the water for hemodialysis by the CHUY hemodialysis center to ensure a better quality of life for patients undergoing this treatment.

FEMS7-0612
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TRANSCRIPTIONAL AND PHYSIOLOGICAL CHARACTERIZATION OF KLEBSIELLA PNEUMONIAE BIOFILM DISPERSED BACTERIA

C. GUILHEN[1], N. CHARBONNEL[1], C. FORESTIER[1], D. BALESTRINO[1]
[1]Universite d'Auvergne, UMR CNRS 6023, Clermont Ferrand, France

Backgrounds

Surface-associated communities of bacteria, known as biofilms, play a critical role in the persistence and the diffusion of bacteria. Biofilm development includes adhesion of bacteria to the surface, formation of microcolonies, maturation with synthesis of an exopolymeric matrix, and dispersion. The final highly regulated step contributes to the dissemination of bacteria in the surrounding environment. Whereas the physiology of planktonic and sessile bacteria is exhaustively documented, very little is known about the properties of biofilm-dispersed cells.

Objectives

We aim to characterize bacteria released from *Klebsiella pneumoniae* mature biofilm.

Methods

Spontaneously biofilm dispersed bacteria were harvested in the effluent of a flow-cell device. Their specific properties were assessed using RNA-seq analysis and phenotypic characterizations (confocal microscopy observations, determination of enzymatic activities, etc.).

Conclusions

Dispersed bacteria were transcriptionally different from both planktonic (logarithmic and stationary phases) and sessile states (7 and 13 hours-old biofilms). In particular, dispersed cells overexpressed a large proportion of genes involved in translation, suggesting a high metabolic activity. This hypothesis was supported by determination of enzymatic activities linked to ATP biosynthesis. In addition, dispersed cells display enhanced abilities to form new biofilm compared to planktonic lifestyle cells, as measured by biomass determination and confocal imaging. Altogether, these data indicate that dispersed bacteria should be regarded as a unique stage in the bacteria lifecycle, transcriptionally different from the other states, with cells harboring mixed properties in between planktonic and sessile forms.

FEMS7-1314
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SPATIAL ORGANIZATION OF MULTI-SPECIES MARINE BACTERIA IN BIOFILM

R. Guillonneau[1], *C. Baraquet*[1], *A. Bazire*[2]
[1]*Université de Toulon, MAPIEM EA 4323, La Garde, France*
[2]*Université de Bretagne Sud- UEB,*
Laboratoire de Biotechnologie et Chimie Marines LBCM- EA 3884- Institut Universitaire Européen de l a Mer IUEM, Lorient, France

Backgrounds

In marine environments, all surfaces submerged are rapidly colonized by bacteria and others microorganisms followed by macro-organisms. These complex communities of organisms formed on inert immersed surfaces have important environmental and economic ramifications. In order to find new antifouling strategies that are usually tested *in vitro* prior to field experiments, multi-species biofilms need to be developed to better apprehend their antifouling efficacy and to better understand mechanisms that govern organisms interactions within more complex biofilms.

Objectives

The aim of this study is to characterise adhesion and biofilm formation of different marine bacterial species, when they are inoculated alone or together and to understand what could be involved in their spatial organization in multi-strains biofilms.

Methods

Static and dynamic biofilm formation were studied to determine the pattern of biofilm formation using confocal laser scanning microscopy. Matrix components were observed using specific fluorescent probes. Bacteria-specific antibodies were used during multi-strains studies.

Conclusions

These marine bacterial strains, whose biofilm grew at different paces and shapes in artificial sea water, produced exopolymers such as eDNA, proteins and exopolysaccharides that can be different according to the strains. Some of them secreted inhibitory supernatants toward others. During multi-strains biofilms experiments, a specific spatial organization was observed with four of these bacteria. Very few studies deal with multi-species biofilm in particular in the marine environment context. This is one of the rare study performed with marine bacteria, whose aim is to understand bacterial spatial organisation within multi-species biofilm.

FEMS7-3229
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ACTIVE MICROBIAL INTERACTIONS INVOLVED IN NITROGEN FATE IN VERTICAL SUBSURFACE CONSTRUCTED WETLANDS TREATING URBAN WASTEWATER

M. Guivernau[1], C. Pelissari[2], C. Ávila[3], J. Garcia[4], P.H. Sezerino[2], F.X. Prenafeta-Boldú[1], M. Viñas[1]
[1]Institute of Agrifood Research and Technology IRTA, GIRO, Caldes de Montbui, Spain
[2]Federal University of Santa Catarina UFSC, GESAD, Florianópolis, Brazil
[3]Catalan Institute for Water Research ICRA, ICRA, Girona, Spain
[4]Technical University of Catalonia UPC, GEMMA, Barcelona, Spain

Backgrounds

Constructed wetlands (CW) are eco-friendly systems, engineered to treat different wastewaters, such as nitrogen enriched sewage. However, microbe-microbe interactions during nitrification/denitrification (NDN) process in the so-called "*nitrification aggregate*" of biofilms, have not been deeply assessed in CW.

Objectives

Two vertical subsurface flow constructed wetlands (VF-CW) treating urban wastewater, were implemented at pilot-scale to boost NDN process by i) unsaturated (UVF) and ii) partially saturated (SVF) configurations.

Methods

Operational Periods I-II (winter-summer) were characterized by a similar hydraulic loading rate and different organic and total nitrogen loading rates. Sand/gravel of top and bottom horizons of CW as well as water influent and effluent, were sampled in triplicate at the end of both periods. Active bacterial/archaeal populations were assessed through DNA/RNA-based 16S-Illumina MiSeq sequencing. Moreover, total/active bacteria, ammonia-oxidizing bacteria (AOB) and archaea (AOA), and typical denitrifiers (16S rRNA, *amoA* and *nosZ*-clade-I genes/transcripts, respectively) were quantified by (RT)-qPCR.

Conclusions

SVF-CW prompted proper redox conditions at bottom layer, that favored active nitrifying-denitrifying populations achieving a more efficient NDN process by increasing nitrogen removal rate concomitant with low nitrite/nitrate accumulation. Noteworthy, biofilm harbored a putative rather stable "*nitrification aggregate*", where AOA/AOB and nitrite oxidizers (NOB) were clearly influenced by seasonal environments linked to oxygen and ammonia limiting conditions. Although ammonia-oxidation was associated to both AOB (*Nitrosospira*) and AOA (*Nitrososphaeraceae*), AOB were clearly overcome by AOA in the more oxidative horizons. Interestingly, AOA were positively correlated with NOB phylotypes belonging to *Nitrobacter*, whilst AOB were negatively correlated with other NOB, *Nitrospira* ($n=6$; $r^2>0.80$ and $r^2>0.63$, respectively).

FEMS7-1084
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE EFFECTS OF DIFFERENT ANTIBIOTICS ON EXPRESSION LEVELS OF USP, SFA/FOC AND CNF1 GENES IN A UPEC STRAIN

D. Gumus[1], F. Kalayci Yuksek[1], E. Yoruk[2], G. Uz[2], E. Celik[2], C. Arslan[2], E.M. Aydın[2], C. Canlı[2], M. Ang Kucuker[1]
[1]Istanbul Yeni Yuzyil University Medical Faculty, Medical Microbiology, Istanbul, Turkey
[2]Istanbul Yeni Yuzyil University Faculty of Arts and Sciences, Molecular Biology and Genetics, Istanbul, Turkey

Backgrounds

The association between environmental factors and bacterial gene expression are well known. The host is main environment for bacteria and they also expose many antibiotics during the treatment of infectious diseases.

Objectives

In this present study, our aim was to investigate possible changes in expression levels of three virulence genes [*sfa/foc* (S and F1C fimbria)*, cnf 1* (cytotoxic necrotizing factor) and *usp* (uropathogenic-specific protein)] in a uropathogenic *E. coli* standard strain (UPEC C7) within the presence of ciprofloxacin, nitrofurantoin and trimethoprim-sulfamethoxazole which are frequently used for the treatment of urinary tract infections.

Methods

The UPEC C7 strain was cultured in tryptic soy broth-TSB (control), TSB+ciprofloxacin (0.016 µg/ml), TSB+nitrofurantoin (3 µg/ml) and TSB+trimethoprim-sulfamethoxazole (0.047 µg/ml). Antibiotics were added according to their sub-minimal inhibition concentrations (MIC). Broth dilution method was used to determine MIC's of antibiotics.

Gene expression levels were determined by q-PCR. The results were evaluated by relative quantitation method to compare the gene expression levels. Tukey's post hoc-test was used for statistical analysis. Each experiment was replicated at least thrice.

Conclusions

We detected statistically significant differences in each gene expression levels for all antibiotics via relative quantification analysis. Fold changes in gene expression was found 0.65, 1.42 and 0.23 for sfa/foc gene; 0.01, 0.01 and 2.84 for cnf1 gene and 0.1, 0.01 and 0.01 for usp gene in the presence of ciprofloxacin, nitrofurantoin and trimethoprim-sulfamethoxazole respectively. In qPCR analysis, melting scores were ranged from 93% to 96%.

This investigation has suggested that antibiotics can play role as an environmental factor which may determine the pathogenicity of bacteria in vivo.

FEMS7-2931
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

HUMAN PROTOZOAN PARASITE DIVERSITY IN WASTE AND TREATED WATER: AN ENVIRONMENTAL METAGENOMICS ANALYSIS

A. Gundogdu[1,2], A. Charyyeva[3], H. Kilic[1], U. Nalbantoglu[2,4]
[1]Erciyes University, Microbiology and Clinical Microbiology, Kayseri, Turkey
[2]Erciyes University, Genome and Stem Cell Center GENKOK, Kayseri, Turkey
[3]Erciyes University, Parasitology, Kayseri, Turkey
[4]Erciyes University, Computer Engineering, Kayseri, Turkey

Backgrounds

Water is a primary source for infections related to digestive system. Especially the consumption of water with insufficient treatment might give rise to the dissemination of the corresponding infectious agents.

Objectives

In this study, wastewater metagenomes of an urban area, a rural area , and a hospital in Turkey were studied using next generation sequencing and bioinformatics analysis, in order to profile the protozoan parasites threatening the human health.

Methods

Samples were collected from wastewater treatment plants before and after treatment and from a hospital's wastewater. Total DNA was extracted using commercial kits and shotgun DNA sequencing was performed using Illumina technology. Obtained metagenomes were filtered for human contamination, and a panel of 80 human parasite genomes were searched using short-read mapping. The total DNA length mapped to each parasite species was nomalized by the genome lengths and the sequencing depth to obtain the relative abundance.

Conclusions

To our knowledge, this is the first parasite profiling study in the environment. Since the treated water is piped to the nearest fresh water sources, the existence of human parasites poses a potential risk to the public utilizing the related natural resources. While the detected sequences belonging to clinically non-reported species might be attributed to the genomic similarities with other unfiltered eukaryotes, they might also stem from the migration of the corresponding protozoa due to the increasing refugee population. Special wastewater treatment eliminating the parasites might reduce the risk of potential transmission of these pathogens from the environment.

FEMS7-2796
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CONTRASTING MICROBIAL COMMUNITIES ALONG PH GRADIENT OF ACID MINE DRAINAGE OF MALANJKHAND COPPER PROJECT, INDIA

A. Gupta[1], A. Dutta[2], J. Sarkar[1], D. Paul[1], M. Panigrahi[3], P. Sar[1]
[1]Indian Institute of Technology Kharagpur, Department of Biotechnology, Kharagpur, India
[2]Indian Institute of Technology Kharagpur, School of Bioscience, Kharagpur, India
[3]Indian Institute of Technology Kharagpur, Department of Geology and Geophysics, Kharagpur, India

Backgrounds

Acid mine drainage is considered as extreme environments for life due to the presence of high concentration of sulfate and metals as a result of bio-oxidation of sulfidic ores. Hence, it becomes a model system for ecologist to understand the microbial composition and their role in biogeochemical cycling.

Objectives

In the present study, the prokaryotic assemblage was elucidated from unexplored acid mine drainage of Asia's biggest open cast, Malanjkhand copper project, India to infer their role in biogeochemistry.

Methods

To assess the composition and structure of microbial community and the environmental factors that shape them, 16S rRNA gene based amplicon sequencing, network analysis, clone library of functional genes involved in C and S cycles, taxon-specific and functional gene based qPCR and physicochemical analysis were performed on AMD sediment and water samples.

Conclusions

The physicochemical parameters showed that the samples were divided into two pH regime low (1.9<pH<4.0) and high (4.0<pH<6.0) with high sulfate concentration. The microbial diversity analysis of the two categorized pH regime showed a distinct microbial assemblages. The abundance of highly acidophilic group involved in Fe/S metabolism were found to be dominated in low pH samples. The qPCR analysis showed that low pH samples harbored less cell density in comparison to high pH samples. In spite of their distinct diversity, the microbial population showed similar physiological functions related to carbon fixation (*cbb*L) and sulfate reduction (*dsr*B) but with varied abundance. This study provides a better insight into microbial composition, their functional roles in biogeochemical cycling and prospect for its bioremediation.

FEMS7-0352
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COMPARATIVE GENOMIC ANALYSIS OF NATIVE PSEUDOMONAS SYRINGAE PLASMIDS BELONGING TO THE PPT23A FAMILY REVEALS THEIR ROLE IN P. SYRINGAE EPIPHYTIC AND PATHOGENIC LIFESTYLES

J.A. Gutiérrez Barranquero[1], *A. de Vicente*[1], *F. Aprile*[1], *G.W. Sundin*[2], *F.M. Cazorla*[1]
[1]*Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora IHSM-UMA-CSIC- Universidad de Málaga, Microbiología, Málaga, Spain*
[2]*Center for Integrated Plant Systems- Michigan State University, Department of Plant- Soil- and Microbial Sciences, East Lansing, USA*

Backgrounds

The pPT23A family of plasmids (PFPs) appears to be indigenous to the plant pathogen *Pseudomonas syringae* and these plasmids are widely distributed and widely transferred among pathovars of *P. syringae* and related species. PFPs are sources of accessory genes for their hosts that can include genes important for virulence and epiphytic colonization of plant leaf surfaces.

Objectives

Further understanding of the evolution of the pPT23A plasmid family and the role of these plasmids in *P. syringae* biology and pathogenesis, requires the determination and analysis of additional complete, closed plasmid genome sequences. Therefore, our main objective was to obtain complete genome sequences of PFPs from three different *P. syringae* pathovars and perform a comprehensive comparative genomic analysis.

Methods

In this work plasmid DNA isolation, purification by CsCl-EtBr gradients, and sequencing using 454 platform, were carried out to obtain the complete sequence of *P. syringae* plasmids. Different bioinformatic tools were used to analyze the plasmid synteny, to identify virulence genes (*i.e.* type 3 effectors) and to unravel the evolutionary history of PFPs.

Conclusions

Our sequence analysis revealed that PFPs from *P. syringae* encode suites of accessory genes that are selected at different levels (universal, interpathovar and intrapathovar). The conservation of type IVSS encoding conjugation functions also contributes to the distribution of these plasmids within *P. syringae* populations. Thus, this study contributes to unravel the genetic bases of the role of PFPs in different *P. syringae* lifestyles.

This work was supported by grants Proyecto de Excelencia, Junta de Andalucía (P07-AGR-02471; P12-AGR-1473) and by Michigan State University AgBioResearch.

FEMS7-0301
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

GENOMIC ADAPTIVE STRATEGIES TO SEVERE METAL-STRESS IN TETRAHYMENA THERMOPHILA: CREATING NEW GENE ISOFORMS UNDER PRESSURE

P. de Francisco[1], A. Martin-Gonzalez[1], J.C. Gutierrez[1]
[1]Universidad Complutense UCM- Facultad Biologia, Microbiologia-III, Madrid, Spain

Backgrounds

High-level stresses can induce "genomic chaos" or genetic instability involving genome shattering and reorganization. The main reported genome alterations induced by stress are: mutations, epigenetics changes and gene copy number variations. *T. thermophila* has five metallothionein (MT) gene isoforms; 3 CdMT genes (*MTT1*, *MTT3* and *MTT5*) and 2 CuMT genes (*MTT2* and *MTT4*).

Objectives

During more than two years, cultures of the this ciliate have been exposed to increasing metal (Cd^{2+}, Cu^{2+} or Pb^{2+}) concentrations, achieving adapted strains to a maximum metal tolerated concentration. In addition, a knockout MTT1 strain (MTT1KO), a knockdown MTT5 strain (MTT5KD) and a double mutant strain (MTT1KO + MTT5KD) have been obtained.

Methods

From all these strains a MT gene copy number quantification has been carried out by quantitative-PCR.

Conclusions

The main conclusions from this study are: 1)- For the first time in ciliates and exclusively in the Cd-adapted strain, we have detected a fast and reversible increase of the copy number (≈ 5-fold) of the *MTT1* gene, involving an over-expression of this gene under extreme Cd-stress. 2)- *MTT5* gene is an essential gene because it is not possible to isolate a stable knockout strain, being the first time that a MT gene is considered as an essential gene. However, a MTT5KD strain has been obtained (copy number reduction of 1n-3n). 3)- In the genome of the MTT5KD strain the presence of two new *MTT1* gene isoforms (*MTT1a* and *MTT1b*) has been detected. Both are originated from homologous recombination between two original *MTT1* gene copies.

FEMS7-0782
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COMPARISON OF BACTERIAL SUCCESSION ALONG THE CHRONOSEQUENCES OF TWO GLACIER FORELANDS OF THE HIGH ARCTIC

H. Gyeong[1], *M. Kim*[1]
[1]*Korea Polar Research Institute, Division of Polar Life Science, Incheon, Republic of Korea*

Backgrounds

Glacier foreland chronosequences offer a model setting for understating ecological succession. Bacterial succession is relatively poorly understood in glacier foreland soils of the High Arctic.

Objectives

We investigated the successional changes in bacterial community composition and diversity along the chronosequences of two glacier forelands (Austre Lovenbreen: AL and Bloomstrandbreen: BS).

Methods

The bacterial communities were analyzed using MiSeq sequencing of 16S rRNA gene. The ANOSIM analysis revealed that bacterial community compositions were significantly shifted along the chronosequences of both AL and BS glacier forelands.

Conclusions

There were directional trajectories in the relative abundance of some of the dominant bacterial phyla throughout succession. Bacteroidetes are more abundant at early successional stages and decreased towards later phases in both regions. Planctomycetes showed a contrasting changing pattern along the chronosequence with increasing relative abundance in AL and vice versa in BL. Bacterial diversity decreased significantly along BS chronosequence, whereas it remained unchanged in AL. Overall, our results indicate that bacterial community compositions were changed in a predictable way along chronosequences of both glacier forelands. However, bacterial diversity and phyla relative abundance showed different patterns along both glacier forelands. This discrepancy between two glacier forelands could be further explained by comparing local environmental conditions and vegetation dynamics in the future.

FEMS7-2898
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ISOLATION AND CHARACTERIZATION OF ELITE INDIGENOUS RHIZOBIA NODULATING PEA (PISUM SATIVUM L.) COLLECTED FROM DIFFERENT BIOCLIMATIC AREA IN TUNISIA

A. Hachana[1,2], *I. Hemissi*[1], *M. Bouraoui*[1], *S. Kouki*[1,2], *N. Abdi*[1], *B. Sifi*[1]
[1]*National Institute of Agronomic Research, Agricultural Sciences and Techniques Laboratory, Ariana, Tunisia*
[2]*National Institute of Agronomic- Carthage University, Science of crop production, Tunis, Tunisia*

Backgrounds

Legumes constitute a very important plant taxon. They play biological, ecological, agronomic and environmental roles Their capacity to fix the atmospheric nitrogen generates a double interest, economic by minimizing nitrate fertilizers applications, and ecological by limiting nitrogenous leakage Legumes contribute also to the improvement of biological balance of rotation in agricultural production systems In spite of their multiple interests, legumes areas in Tunisia are continuously regressing, leading a decrease in the production particularly in pea culture The extension of this culture is limited by its extreme sensibility to biotic and abiotic stresses and the absence of specific and efficient *Rhizobium* strains

Objectives

The objective of this study is the isolation and selection of elite indigenous rhizobia to improve the productivity of pea crop

Methods

In this study, indigenous *Rhizobium* strains were isolated from nodules of pea roots or directly from soil collected from different regions of Tunisia These isolates were morphologically characterized by the Gram test and the study of their growth in alkaline media The selected strains were also biochemically characterized for their assimilation of different carbonate substrates Then, they were screened for their ability to fix atmospheric nitrogen and solubilize inorganic phosphate in Pikovskaya medium and the potassium in Aleksandrov medium

Conclusions

The results obtained showed a large variability in the characteristics of *Rhizobium* strains and their performance for nitrogen fixation and phosphorus and potassium solubilisation in dependence on their origins This shows that the *Rhizobium*-pea symbiosis efficiency is largely dependent on the pedoclimatic conditions of the crop areas

FEMS7-2771
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE EFFECT OF NITROGEN FERTILISATION AND PLANT GENOTYPE ON THE FUNCTION AND COMPOSITION OF MICROBIAL COMMUNITIES IN THE RHIZOSPHERE OF FIELD GROWN BRASSICA NAPUS

C. Hale[1], *H. Schäfer*[1], *G. Teakle*[1], *G. Bending*[1]
[1]*University of Warwick, School of Life Science, Coventry, United Kingdom*

Backgrounds

Plant roots and adhering soil (the rhizosphere) host a diverse and active microbial community. The composition of bacteria and microbial eukaryotes, inhabiting the rhizosphere can have significant impacts on plant health through a range of direct and indirect interactions. A wide range of factors can potentially influence the distribution and abundance of microbial taxa in the rhizosphere.

Objectives

The aims of this study were to determine the roles of nitrogen (N) fertilisation and the genotype of *Brassica napus* in shaping the diversity, composition and function of microbial communities in the rhizosphere and surrounding bulk soil, and the implications for plant health.

Methods

Rhizosphere and soil samples were taken from field plots at the green bud stage. We used targeted amplicon sequencing to determine the structure of bacterial, protist and fungal taxa respectively. Furthermore we used PICRUSt, NEMAGuild and FUNGuild to predict whether the shifts in community composition impacted function.

Conclusions

Plant genotype had no significant effect in shaping the microbial community of the rhizosphere. N fertilisation significantly altered the soil microbial eukaryote communities, but had no significant effect on the bacterial soil community. Community composition of all groups were affected by N fertilisation in the rhizosphere. PICRUSt analysis indicated an increase in antibiotic genes in the low N rhizosphere communities. NEMAGuild analysis indicated an increase of nematode plant parasites in the rhizosphere of the high N treatment. FUNGuild analysis indicated an increase of pathotrophs in the rhizosphere, however N treatment had no effect on fungal functional guild in the soil or rhizosphere.

FEMS7-1799
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE ROLE OF QUORUM SENSING SIGNALS ON THE INTERACTIONS BETWEEN VIBRIO CHOLERAE AND CHIRONOMIDS MICROBIOTA

M. Halpern[1], *N. Atamna-Ismaeel*[1], *B. Hammer*[2]
[1]*University of Haifa, Biology and Environment, Haifa, Israel*
[2]*Georgia Institute of Technology, School of Biology, Atlanta, USA*

Backgrounds

Vibrio cholerae causes the fatal cholera diarrhea and is a natural inhabitant of aquatic ecosystems. We commonly isolate *V. cholerae* along with other bacterial species from all four life stages (eggs, larvae, pupae and adults) of chironomid insects. *V. cholerae* secretes Haemagglutinin/Protease (HAP) that degrades the gelatinous matrix of chironomid egg masses, likely acquiring nutrients and consequently preventing hatching. HAP is activated by Quorum Sensing (QS), a bacterial cell-cell communication process in which accumulation of extracellular chemical molecules (called autoinducers, AIs) triggers an intracellular signal transduction cascade.

Objectives

Our aim was to define the role of QS AI signals produced by chironomids microbiota on the production of HAP by *V. cholerae*, and to characterize the complex relationships of chironomids, *V. cholerae,* and other members of the egg mass microbial community

Methods

To study the role of QS signals we used *V. cholerae* bioluminescence reporter strains (QS-proficient O1 El-Tor wild type and QS-deficient mutants).

Conclusions

The results demonstrated that *V. cholerae* responds to AIs produced by other members of the chironomid bacterial consortium by expressing the *hapA* gene. The egg mass microbiota may use the degraded gelatinous matrix of the egg mass for their own growth. By doing so, these other species may in turn control the population levels of *V. cholerae* in the egg mass. So too *V. cholerae* supports the maintenance of endogenous bacteria in the egg mass by secreting HAP. Understanding *V. cholerae* QS in the insect system may help uncover the interactions between this pathogen and the human gut.

FEMS7-0882
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ABUNDANT MICROORGANISMS IN DIGESTERS OVERLOOKED BY AMPLICON SEQUENCING DUE TO PRIMER BIAS

L. Hao[1], R. Kirkegaard[1], M. Dueholm[1], S. McIlroy[1], J. Chenjing[1], M. Albertsen[1], P. Nielsen[1]
[1]Center for Microbial Communities, Department of Chemistry and Bioscience- Aalborg University, Aalborg Ø, Denmark

Backgrounds

Amplicon sequencing of the 16S rRNA gene is often applied to study the presence and population dynamics of uncultured bacteria in anaerobic sludge digesters. Such studies can give important information about novel bacteria and how they may play important roles in this complex engineered microbial system. However, primer bias can cause severe problems, and sometimes abundant and novel microorganisms may be completely overlooked.

Objectives

The microbiome in a full-scale mesophilic sludge digester at a Wastewater Treatment Plant (WWTP) was studied by amplicon and shot-gun sequencing, to investigate the influence of PCR primer choice.

Methods

Three primer-sets targeting V1-3, V3-4 and V4 region of the 16S rRNA gene, respectively, were used for amplicon sequencing, and the results were compared with the metagenomic data.

Conclusions

The results showed that the relative read abundance of some novel microbial phylotypes was seriously affected by the choice of primers. Microbial phylotypes affiliated to the candidate phylum Acetothermia demonstrated 2.3~6.7% relative abundance according to metagenomic analysis. This was, however, underestimated by two orders of magnitude when V1-3 and V3-4 primers were used. V4-primer mediated amplicon sequencing, nevertheless, demonstrated similar results to the metagenomic analysis, with 3.5~10.1% relative read abundance detected in various samples. The detection of several other candidate phyla was also significantly influenced by primer choice, including Cloacimonetes and Fermentibacter.

A survey was conducted on these overlooked microorganisms in digesters at 19 Danish WWTPs. The results showed that several of these phylotypes were among the most abundant bacteria in most digesters, indicating their significance to the digester performance.

FEMS7-2463
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBE-MEDIATED PLANT-SOIL FEEDBACK AS A POSSIBLE DRIVER OF PRIMARY SUCCESSION

L. Harantova[1], E. Kuťáková[2], P. Kohout[1], P. Baldrian[1]
[1]Institute of Microbiology - Academy of Sciences of the Czech Republic, Ecology, Prague, Czech Republic
[2]Institute of Botany- Czech Academy of Sciences, Department of population ecology, Prague, Czech Republic

Backgrounds

Primary succession of plants is a fundamental ecological process, however, it is soil microorganisms that first colonize barren substrate. They change its physico-chemical properties, increase nutrient availability and facilitate plant establishment and growth. Plants in turn shape their associated soil microorganisms and modulate soil properties. This plant-soil feedback (PSF) affects subsequent performance and competitive ability of plants and can thus contribute to successional dynamics of plant communities.

Objectives

Using microcosm experiment, we studied soil microbial communities of three early- and three mid-successional plant species. Our main objective was to clarify whether early- and mid-successional species harbour different bacterial and fungal communities, what environmental factors mediate the differences, and how soil microbes contribute to PSF during successional transition.

Methods

Composition of soil bacterial and fungal communities was studied using next-generation sequencing of 16S and ITS amplicons. After one growth season, plant-soil feedback was assessed as plant performance in soil of their own as compared to soil from other five species.

Conclusions

Our results showed that soil microbial communities are both plant-species- and successional-stage-specific, with the effects being more pronounced in the case of fungi. Bacterial community composition is mostly driven by organic carbon content, whereas fungi respond also to soil pH and potassium. Early-successional plant species exhibited more positive plant-soil feedback than mid-successional ones.

FEMS7-2333
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

POTENTIAL FOR EPIGENETIC MODIFICATIONS OF THE GENOME TO INFLUENCE VIBRIO VULNIFICUS VIRULENCE

V. Harwood[1], *J. Conrad*[1]
[1]*University of South Florida, Integrative Biology, Tampa, USA*

Backgrounds

BACKGROUND. The opportunistic pathogen *Vibrio vulnificus* is a natural bacterial inhabitant of coastal waters. No definitive virulence factors outside of a capsule have been identified, yet *V. vulnificus* strains display differential virulence. Epigenetic modifications such as DNA methylation can cause changes in gene expression, and may play a role in virulence. Three methyltransferases, which methylate DNA at specific sequences, have been identified in the genome of *V. vulnificus* CMCP6.

Objectives

OBJECTIVES: We hypothesize that (1) epigenetic regulation of gene expression, including DNA methylation, may contribute to a highly virulent phenotype, and (2) that environmental conditions influence gene expression via methylation patterns. Our objective was to sequence the methylome of *V. vulnificus* CMCP6 grown under varying conditions to identify modifications that may control gene expression, and to compare the methylome under varying environmental conditions.

Methods

METHODS. We sequenced the genome of *V. vulnificus* CMCP6 using single molecule real time sequencing (SMRT) in cultures grown in human serum (HS) or sterile natural seawater (SW). A *de novo* assembly of the genome was constructed and methylation was assessed using PacBio's modification and motif analysis pipeline.

Conclusions

CONCLUSIONS. The analysis revealed two distinct 6-methyladenine (6mA) motifs, GmATC and GGmAN$_9$TGGC/GCCmAN$_9$TCC, in both SW and HS treatments, and several other putative motifs. Two 4-methylcytosine (4mC) motifs (mCSN$_5$G and mCWGNNVNG) were detected in HS, but not SW cultures. Genes related to oxidative stress response had several methylation motifs, suggesting regulation by methylation. Further analysis and transcriptomic data will elucidate the effect of methylation on gene expression under varying environmental conditions.

FEMS7-2878
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INFLUENCE OF TRIPARTITE INTERACTIONS ON PHOSPHORUS ACQUISITION UNDER DROUGHT STRESS IN STERILIZED AND NON-STERILIZED SOILS.

L. Hassani Rezaei[1]
[1]*Department of Agriculture, Yadegar-e-Imam Khomeini(RAH) shahre rey Branch, Islamic Azad University, Tehran, Iran*

Backgrounds

Nitrogen fixers when used with arbuscular mycorrhizal fungi which improve absorption of nutrients from soil, increase N and phosphorus content of the plants particularly in low phosphorus soils.

Objectives

Comparison of phosphorus acquisition by alfalfa plants in the sterilized and non-sterilized soils under different moisture conditions.

Methods

The experiment was under green house conditions. Symbiotic association effects between VAM fungi, Rhizobium bacteria and alfalfa plants under 10, 15 and 22 percentage moisture levels was studied in sterilized and non-sterilized soils. The trial was laid out by factorial experiment in the form of Complete Randomized Block Design with 5 replications.

Conclusions

The main effect of moisture levels ,VAM inoculation and also Double interaction of rhizobium Î moisture level significantly increased Phosphorus uptake at 1% probability level in the both sterilized and non-sterilized soils. The main effect of rhizobium bacteria on phosphorus nutrition was significant in non-sterilized soil at 5% level but it wasnot significant in the other soil. The synergistic effect between VAM and rhizobium had not significant effect on mentioned parameter in the both soils. Double interaction of VAM Î moisture level and also triple interaction of VAM Î rhizobiumÎmoisture level became significant on phosphorus uptake at 1% level in the sterilized soil but they were not significant in the non-sterilized soil.

FEMS7-1130
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

QUORUM SENSING IS INVOLVED IN ADHESION AND BIOFILM FORMATION OF SHEWANELLA WOODYI

M. HAYEK[1], *C. Baraquet*[1], *Y. Blache*[1], *M. Molmeret*[1]
[1]*University of Toulon, MAPIEM LABORATORY, Toulon, France*

Backgrounds

Quorum sensing (QS) or cell-to-cell communication is a process by which bacteria produce and detect signal molecules and thereby coordinate their behavior in a cell-density dependent manner. Two main QS systems can be distinguished: the acylhomoserine lactone (AHL) and the autoinducer-2 (AI-2). The sequenced genome of the marine bioluminescent *Shewanella woodyi* MS32 contains genes coding for these two main QS systems.

Objectives

The objectives of this work are to identify the QS communication systems present in *S. woodyi* and to determine its role in adhesion and biofilm formation.
The aim of this work is to find molecules that interfere with QS processes to inhibit adhesion and biofilm formation of *S. woodyi*.

Methods

In this study, we used the bacterial biosensor assay and the high resolution liquid chromatography mass spectrometry (LCMS) to identify the active QS systems and to specify the AHL (s) synthesized by *S. woodyi.* Luminescence Fluorescence and Crystal violet assay were also used to select molecules that inhibit adhesion and biofilm formation by interfering with the QS system of this bacterium.

Conclusions

For the first time, the active QS systems in *S. woodyi* and its role in adhesion and biofilm formation was determined. *S. woodyi* synthesizes an C8-HSL and the AI2 QS molecule. Some exogenous AHLs and other molecules known to have an effect on QS, can influence the luminescence of this bacterium, in particularly oxo and hydroxy derivatives, and reduce adhesion and biofilm formation. Mutation of putative QS genes should help determining the precise role of QS in biofilm formation.

FEMS7-3092
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

AGRICULTURAL BIOSTIMULANTS DIFFERENTIALLY AFFECT THE BACTERIAL, FUNGAL AND ARCHAEAL COMMUNITIES OF SOIL: AN ILLUMINA SEQUENCING BASED-ANALYSIS

E. Hellequin[1], C. Monard[1], O. Klarzynski[2], M. Henriot[2], F. Binet[1]
[1]Universite de Rennes 1, CNRS-UMR 6553 Ecobio, Rennes, France
[2]Bio3G l'Agronome, research and development, Merdrignac, France

Backgrounds

To respond to the increasing demand for food and in the context of ecological transition, the use of agricultural biostimulants (BS) is becoming a common practice for sustainable agriculture. These BS can either be applied to plants or soils to enhance plant nutrition and growth. However, the mechanisms by which soil BS improve soil biological functioning and increase crop yields are still ignored. We thus focused our research on a soil BS, developed by the BIO3G Company to improve the degradation of crop residues, limit leaching of nutrients and release nutrients faster for the next crop.

Objectives

We hypothesize that changes in the microbial communities induced by the BS are responsible for the increase of crop residues mineralization. The objectives were to (i) determine the impact of BS on straw mineralization by soil microorganisms, (ii) identify whether the action was due to the inoculation of microorganisms and/or the stimulation of soil indigenous microorganisms, (iii) identify the soil microbial communities recruited in presence of BS.

Methods

Soil samples with straw were incubated with or without BS and the soil mineralization was monitored for 49 days. At the end of the incubation, changes in both microbial biomass and bacterial, fungal and archaeal diversity were determined by using a metabarcoding approach.

Conclusions

We demonstrated that the soil BS stimulated soil mineralization and increased the microbial biomass. Changes in bacterial and fungal community structures were observed due to the stimulation of soil indigenous microorganisms rather than an inoculation of specific microorganisms.

FEMS7-2962
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

NEW FUNGAL COMMUNITY WITH DISTINCT SPECIES COMPOSITION: FILAMENTOUS FUNGI ASSOCIATED WITH REED GALL INQUILINES

P. Heneberg[1], P. Bogusch[2], A. Astapenková[2], D. Šimčíková[1], A. Čmoková[3], M. Kolařík[3]
[1]Charles University, Third Faculty of Medicine, Prague, Czech Republic
[2]University of Hradec Králové, Faculty of Science, Hradec Králové, Czech Republic
[3]Charles University, Faculty of Science, Prague, Czech Republic

Backgrounds

Backgrounds: Monotypic stands of common reed (*Phragmites australis*) and the reed-gall-associated insect assemblages are distributed worldwide. However, fungi associated with these assemblages have never been characterized in detail.

Objectives

Objectives: We aimed to characterize the newly identified fungal community and perform a large-scale experimental verification of the infectivity of the species found.

Methods

Methods: Here we examined over 10,000 individuals of 14 species of immature reed gall inquilines from the order Hymenoptera collected at 34 sampling sites across Europe for the presence of fungal infections. We cultivated the fungi on basal as well as on selective media. To confirm their pathogenicity, the representative spectrum of newly identified strains was allowed to sporulate and the spores were inoculated on larvae of the eudominant reed gall inquiline, *Pemphredon fabricii*. We then counted the numbers of larvae, which died due to the infection or which managed to survive and fully develop to imagines, and recorded differences in a time needed to develop under fungal stress.

Conclusions

Conclusions: We found that the Hymenoptera occupying the reed galls serve as previously unknown microhabitat hosting abundant assemblage of filamentous fungi dominated by *Penicillium buchwaldii* and *Aspergillus pseudoglaucus*, but hosting also classical entomopathogenic species, such as *Lecanicillium attenuatum*. Fungal infections were an important driver of the abundance of reed gall-associated aculeate hymenoptera. Infections of generalist host species were more frequent than those of reed gall specialists, suggesting that suboptimal conditions decrease the immunocompetence of nonspecialized species, which only occasionally nest in reed.

FEMS7-2665
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

OMICS APPROACH TO PROBE THE MECHANISM FOR INTERACTION BETWEEN CHLORELLA VULGARIS AND BACTERIA

J. Heo[1,2], D.H. Cho[1], H.S. Kim[1,2]
[1]Korea Research Institute of Bioscience & Biotechnology, Cell Factory research center, Daejeon, Republic of Korea
[2]University of Science and Technology, Green Chemistry and Environmental Biotechnology, Daejeon, Republic of Korea

Backgrounds

Over the past 20 years, people have studied the relationships between algae and bacteria from different perspectives, including the physical, biological, environmental and chemical processes involved. Recent studies on co-cultivation of algae with growth enhancing bacteria showed that bacteria enhance algal growth rate by at least 10%. Our previous studies have proved that selective infestation of growth promoting bacteria in the phycosphere of algae would lead to a cumulative increase in biomass and lipid productivity.

Objectives

The metabolomics study of interactive mechanisms between microalgae and bacteria could help improve algal biomass productivity in microalgae production processes. Moreover, the omics approach to study microbial ecology has transformed our understanding of microbial communities and their environment. In this study, the major objective is to examine the mechanism for interaction between *Chlorella vulgaris* and its associated bacteria.

Methods

Chlorella vulgaris OW-01 (NCBI accession number JQ664295) used in this study were isolated from wastewater. Axenic unialgal culture of *C. vulgaris*(ACV) were grown in BG11 medium for 14days (25℃, 150μmol/m²/s). After cultivation, the culture broth of ACV was filtered through a 0.2μm pore size bottle-top filter system. This filtered culture broth was utilized for microalgae growth-promoting bacteria cultures. Axenic *chlorella vulgaris* was co-cultured with bacterial metabolite fraction which were obtained from the bacterial culture supernatant.

Conclusions

This study suggests that the omics approach would help answer some basic questions on algal-bacterial association.

FEMS7-2753
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CONDUCTIVITY RATHER THAN OXYGEN LEAKAGE DETERMINES ABUNDANCE AND COMPOSITION OF AMMONIA OXIDIZERS ON TYPHA LATIFOLIA ROOTS

E. Hernandez-del Amo[1], *G. Ramis*[1], *F. Gich*[1], *L. Bañeras*[1]
[1]*Universitat de Girona, Institute of Aquatic Ecology, Girona, Spain*

Backgrounds

Oxidation of ammonia in water and sediments is highly dependent on the availability oxygen. Emergent macrophytes, such as *Typha latifolia*, increase oxygen concentrations in the rhizoplane due to continuous oxygen leakage from roots, thus generating an aerobic microenvironment in close contact to the root surface. However, the impact of oxygen leakage on the abundance and diversity of nitrifiers has not been extensively quantified.

Objectives

The aim of this study was to quantify to what extent oxygen permeability on *Typha latifolia* roots affected the spatial distribution of ammonia oxidizing archaea (AOA) and bacteria (AOB) in an estuarine salinity gradient.

Methods

Oxygen leakage at different parts of root hairs was estimated as potential diffusion rates after measuring oxygen micro-profiles at the root surface. AOA and AOB diversity and abundance in root sections were inferred by barcode amplicon Illumina sequencing of the 16S rRNA gene, and quantitative PCR of *amoA* and 16S rRNA genes.

Conclusions

AOA and AOB abundances increased significantly in high conductivity areas (>10,000 mS/cm). The basal sections of root hairs contained a higher abundance of ammonia oxidizers, despite the low oxygen diffusion rates measured. Relative abundance of AOA was higher compared to AOB both in the root surface and the sediment adjacent to it. However, AOB/AOA increased in the root surface for some specific taxa. Overall, we were able to show that water conductivity and plant roots exerted a selection effect on ammonia oxidizers, although this effect may be limited to small portions of the root surface due to changes on oxygen leakage.

FEMS7-3178
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

IN VITRO CELECOXIB SUPPLEMENTATION IMPACTS THE FUNCTIONAL CAPACITIES OF THE GUT MICROBIOTA

E. Hernandez-Sanabria[1], E. Heiremans[1], M. Calatayud Arroyo[1], B. Roman[2], S. Mangelinckx[2], L. Leclercq[3], J. Snoeys[3], T. Van de Wiele[1]
[1]Ghent University, Center for Microbial Ecology and Technology CMET, Gent, Belgium
[2]Ghent University, Department of Sustainable Organic Chemistry and Technology, Gent, Belgium
[3]Janssen Pharmaceutica NV, Janssen Research & Development, Beerse, Belgium

Backgrounds

Alterations on inflammatory pathways lead to aberrant expression of cyclooxygenase-2 (COX-2) in colon carcinogenesis (CRC). The efficacy of COX inhibitors (coxibs) for successfully reducing CRC recurrence further confirmed the key role of COX-2. Alas, continuous COX-2 inhibition may increase the risk of a cardiovascular event. Currently, little information is available on how inter-individual variations in colon microbiota impact coxib disposition and overall celecoxib disposition.

Objectives

This project evaluated the effect of clinical concentrations of celecoxib on the in vitro colon microbiota. We determined the baseline microbiota activities and metabolic response, to reveal whether microbial drug metabolism impacts the conversion process.

Methods

We conducted in vitro batch culture experiments, assessing the potential of human faecal microbiota for metabolising celecoxib. Faecal slurries from four volunteers were supplied with 100 mg/ml of celecoxib and anaerobically incubated for 16h, to simulate the transit time of the proximal colon. Short-chain fatty acids (SCFAs) were considered benchmarks of gut microbial functionality and determined by gas chromatography. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine celecoxib recovery. Total RNA was applied to perform qRT-PCR of the bacterial 16S rRNA gene and to evaluate the metabolically active population.

Conclusions

Our results indicate that celecoxib shifts in vitro fermentation, in a donor-dependent manner. Celecoxib significantly decreased total SCFA and butyrate ($P < 0.001$), but not copy number of 16S rRNA gene in all donors. Microbial-derived SCFA, such as butyrate, may fuel proliferation of cancer-initiated epithelial cells. This study will provide information about the microbiota interplay on the efficacy of colon-targeted coxibs.

FEMS7-1620
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CHALLENGE TO DISCOVER THE ANCIENT MICROBIOTA IN HOLOCENE SEDIMENTS OF THE EL PORTALON CAVE AT THE SIERRA DE ATAPUERCA (BURGOS) BY HIGH-THROUGHPUT SEQUENCING

M. Hernandez[1], D. Rodríguez-Lázaro[2], N.M. Quijada[1], G. Kay[3], A. Pérez Romero[4], C. Valdiosera[5], E. Iriarte[4], J.M. Carretero[4]
[1]Instituto Tecnológico Agrario de Castilla y Leon ITACyL, Laboratory of Molecular Biology and Microbiology, Valladolid, Spain
[2]University of Burgos, Department of Biotechnology and Food Science. Area of Microbiology, Burgos, Spain
[3]University of Warwick, Microbiology and Infection Unit- Warwick Medical School, Coventry, United Kingdom
[4]University of Burgos, Laboratory of Human Evolution, Burgos, Spain
[5]La Trobe University, Department of Archaeology and History- Faculty of Humanities and Social Sciences-, Melbourne, Australia

Backgrounds

The Archaeological Sites of “Sierra de Atapuerca” are located in the northeast sector of the Cenozoic Duero Basin, north-central Iberian Peninsula, very near to Burgos (Spain) and has been included in the UNESCO list of World Heritage Sites. It was occupied by hominids from the Early Pleistocene to modern man and it is continuously being excavated every year during a limited period of time in summer.

Objectives

The present study is focused on the microbial analysis of Late Pleistocene and Holocene stratigraphic levels of the El Portalón cave, in the Sierra de Atapuerca, by high-throughput sequencing. We have analysed samples from the Late Pleistocene levels (30.000 years BP) and throughout the Holocene, covering the Neolithic, Chalcolithic, Bronze Age and Roman period levels (6000 to 2.000 years BP).

Methods

Total DNA was extracted from sediments, taken special measures to avoid cross contamination. Libraries were prepared using TruSeq Nano DNA Library Preparation Kit (Illumina) and sequenced by synthesis using a MySeq platform. Quality-filtered sequences were analyzed by using Kraken for prokaryotic reads, and eukaryotic DNA by Blast algorithm and visualized using Megan.

Conclusions

Classical microbiological determination performed in some of the sediments allowed to grow bacteria corresponding to soil microbiota naturally present. Species as *Alteromonas* or *Pseudomonas* were generally present in the sediments. Besides, the DNA extraction yield highly compromised the results, and very diverse bacteria at low levels were identified, particularly those bacteria osmotolerant and facultative anaerobic in the different strata.

FEMS7-0769
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

FECB (FUNCTIONAL ENCYCLOPEDIA OF CYANOBACTERIA): TOWARDS A MULTI-SCALAR UNDERSTANDING OF BIOLOGICAL CARBON AND NITROGEN CYCLING OF PHOTOTROPHIC CONSORTIA***** Withdrawn by author, Not attending, withdrawn from session pv14 ***

M. Connolly[1], J. Garcia[2], D.E. Culley[3], E.M. Zink[4], S.O. Purvine[4], D. Hu[4], A. Dohnalkova[4], T. Woyke[5], M. Barton[5], D. Coleman-Derr[6], T.D.R. Glavina[5], S. Lea[4], G. Orr[4], E. Robinson[4], T.R. Northen[5], P.E. Larsen[7], K.P. Keegan[7], S.G. Tringe[5], R.W. Castenholz[8], M. Hess[9]
[1]University of California Davis, Microbiology Graduate Group, Davis, USA
[2]University of California Davis,
Biochemistry- Molecular- Cellular and Developmental Biology Graduate Group, Davis, USA
[3]Pacific Northwest National Laboratory, Chemical and Biological Process Development, Richland, USA
[4]Pacific Northwest National Laboratory, Environmental Molecular Sciences Laboratory, Richland, USA
[5]Lawrence Berkeley National Laboratory, DOE Joint Genome Institute, Walnut Creek, USA
[6]US Department of Agriculture, Agricultural Research Services, Albany, USA
[7]Argonne National Laboratory, Biosciences Division, Lemont, USA
[8]University of Oregon, Department of Biology, Eugene, USA
[9]University of California Davis, Animal Science, Davis, USA

Backgrounds

Cyanobacteria are photosynthetic bacteria found in most types of illuminated environments. Their global biomass has been estimated to be ~3×10^{14} g carbon and their key role in global carbon and nitrogen cycling has been widely accepted. Despite the ecological importance and the significant efforts by the scientific community to advance our understanding of this important phylum, most of the research in this field is performed on pure cultures – which represent only a few percent of the organisms in an environmental sample. Very little is known about how members of the phylum cyanobacteria affect and respond to changes in complex biological systems.

Objectives

In the project presented here we selected photosynthetic consortia based on their culture maintenance condition and their collection site from a total of over 1,200 samples that are currently grown in our laboratories. We subjected these cultures to a diverse set of omics and imaging techniques to define the molecular processes that facilitate carbon and nitrogen sequestration by these phototrophic consortia and the individual organisms and interrelationships that contribute to them.

Methods

Methods used during this study included: 16S rRNA sequencing, metagenomics. metabolomics, metaproteomics, and FISH

Conclusions

Here we present first results from this study that was funded by the U.S. Department of Energy (DOE) as part of the FICUS Program at the Joint Genome Institute and the Environmental Molecular Sciences Laboratory, two User Facilities managed by the DOE Office of Science. We anticipate that this study will provide new insights into the growth requirements of the microorganisms that form phototrophic consortia, ultimately facilitating their isolation and axenic cultivation.

FEMS7-2951
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EXPLORING THE DRIVERS OF ANTIMICROBIAL RESISTANCE WITHIN THE ENVIRONMENT

J. Holden[1], E. Wellington[1], W. Gaze[2], L. Zhang[2], A. Singer[3], A. Mead[4]
[1]University of Warwick, School of Life Sciences, Coventry, United Kingdom
[2]Exeter University, Life Sciences, Truro, United Kingdom
[3]Centre for Ecology and Hydrology, Microbial Ecology, Wallingford, United Kingdom
[4]Rothamsted Research, Computational and Systems Biology, Harpenden, United Kingdom

Backgrounds

Persistent administration of antibiotics has placed enormous selective pressure on bacterial species. As a result, we have observed a significant rise in the number of antimicrobial resistant bacteria. Many resistance genes are located on mobile genetic elements and therefore resistant bacteria are capable of transferring genes conferring resistance to human and animal pathogens via horizontal gene transfer.
The (mis-)use of antibiotics alone is not solely responsible for the spread of antimicrobial resistance across bacterial populations. Effluent from hospital/communities, as well as agricultural runoff contain sub-lethal levels of antibiotics and resistant bacterial species; and whilst mitigation strategies are in place to reduce microbial load eg. wastewater treatment plants, these processes have been recently shown to positively select for resistant determinants that are released into the environment.

Objectives

Since bacterial communities are shaped by a complex array of evolutionary, ecological and environmental factors, it is important to take into account not only point sources, but also additional environmental variables when analyzing the drivers of antimicrobial resistance.
We aim to investigate the risk factors associated with the spread of antimicrobial resistance within the Thames catchment.

Methods

River sediment was collected from 69 sites within the Thames catchment. DNA was purified from each sediment and used for next generation sequencing data of resistance genes. GIS spatial data on land use, water quality analyses and rainfall data are used to predict the drivers of antimicrobial resistance.

Conclusions

Preliminary analyses suggest wastewater treatment plants as well as agricultural practices strongly influence the resistance loads within the Thames catchment.

FEMS7-1914
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

HYPORHEIC ZONE: A HARBOUR FOR NOVEL MICROBIAL IBUPROFEN DEGRADERS

C.R. Njeru[1], M. Posselt[2], M.A. Horn[1]
[1]Leibniz Universität Hannover, Institute of Microbiology, Hannover, Germany
[2]Stockholm University, Department of Environmental Science and Analytical Chemistry, Stockholm, Sweden

Backgrounds

Presence of the widely used pharmaceutical Ibuprofen in effluents and receiving waters is attributed to inefficient removal by conventional wastewater treatment plants. Potential total removal via biodegradation in the hyporheic zones (HZ) of receiving rivers downstream of discharge sites has been hypothesized.

Objectives

A HZ downstream of an impacted river was evaluated for its performance in removal of ibuprofen using two sets of oxic HZ sediment microcosms spiked with ibuprofen only (5, 40, 200 and 400 µM), or ibuprofen and 1 mM acetate in batch experiments.

Methods

We applied oxic microcosm incubations coupled to amplicon imllumina sequencing and qPCR of 16S rRNA genes and transcripts to adress hitherto unknown microbes associated with the degradation of the model micropollutant ibuprofen.

Conclusions

Ibuprofen was completely removed in non-sterile relative to autoclaved sediments indicating microbial degradation. In refed microcosms, biodegradation occurred in the absence and presence of supplemental acetate within 24 hours following initial incubation of 11 and 16 days respectively. Carboxy- ,1-, 2-, and 3-hydroxy-ibuprofen were identified metabolites. The microbial communities involved were evaluated using Quantitative real-time PCR and time resolved triplicate amplicon Illumina MiSeq sequencing targeting 16S rRNA genes and transcripts. The analyses revealed similar copy numbers of 16S rRNA gene or transcripts between non-spiked controls and treatments. Increased relative abundances of the phyla *Proteobacteria*, *Acidobacteria* and *Actinobacteria* in treatments with compared to those without ibuprofen were observed. *Proteobacteria* was the dominant phylum with *Alpha-, Beta-* and *Deltaproteobacteria* being the most active as indicated by RNA based analyses. Hitherto unclassified species of *Acidobacteria* subgroup 6 and genera *Hyphomicrobium* and *Sphingomonadaceae* were linked to ibuprofen degradation.
Overall, the results unravel the HZ as a reservoir of hitherto unknown microbial communities associated with turnover and degradation of such micropollutants; a prominent ecological service in the natural self-purification of streams.

FEMS7-2112
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL CONTAMINATION OF CURRENCY NOTES AND COINS IN CIRCULATION IN UNITED ARAB EMIARTES (UAE)

I. Mukhtar[1], M.E.M. Mohamed[1], R.M.A. Hassan[1], A.K.S.A. Ibrahim[1], M. Magaogao[2], A. Hossain[2]
[1]Ras Al Khaimah Medical and Health Sciences University, Medicine, Ras Al Khaimah, United Arab Emirates
[2]Ras Al Khaimah Medical and Health Sciences University, Medical Microbiology and Immunology, Ras Al Khaimah, United Arab Emirates

Backgrounds

Currency notes and coins are items which are most frequently passed from hand to hand. Studies from different countries of the world have shown that currency notes and coins carry different bacterial and fungal species, including pathogenic strains.

Objectives

This study was designed to screen all 11 units of currency notes and coins in circulation in UAE for presence of bacterial species. In addition we also intended to determine the antibiotics resistance profile and biofilm forming potentials of selected dominant bacterial species isolated from currency notes and coins.

Methods

We screened a total of 55 currency notes and coins (5 of each units), which were collected from people engaged in various trades. Saline soaked sterile swab sticks were used to collect the bacterial samples from notes and coins, grown on blood agar and identified by standard microbiology procedures. Five dominant bacterial species of each note and coin were analyzed for antibiotic resistance by disk diffusion assay and biofilm production by crystal violet dye binding spectrophotometric assay.

Conclusions

The data obtained in this study indicates that UAE currency notes and coins carry antibiotic resistant, biofilm forming potential pathogens and may thus serve as a vehicle of transmission of these bacterial species. This is the first report of analyzing UAE currency notes and coins for carriage of bacteria.

FEMS7-0998
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

METAGENOMICS SURVEY OF LIMESTONE CAVE IN TAIWAN

B.M. Hsu[1]
[1]*National Chung Cheng University, Earth and environmental sciences, Chiayi, Taiwan R.O.C.*

Backgrounds

The limestone of Narro-Sky in Tainliao, Taiwan is of Pleistocene reef limestones interbedded in clastic layers that covered the Takangshan anticlines. Understanding how microbial relative abundance was changed in response to changes of environmental factors may contribute to better comprehension of roles that microorganisms play in altering the landscape structures.

Objectives

In this study, microorganisms growing on the wall of limestone, in the water dripping from the limestone wall and of soil underneath the wall were collected from different locations where the environmental factors such as daytime illumination, humidity, or pH are different.

Methods

Next generation sequencing (NGS) was carried out to examine the compositions and richness of microbial community. The metagenomics were clustered into operational taxonomic units (OTUs) to analyze relative abundance, diversities and principal coordinates analysis (PCoA).

Conclusions

Our results showed the soil sample has the highest alpha diversity while water sample has the lowest. Four major phyla, which are Proteobacteria, Acidobacteria, Actinobacteria, and Cyanobacteria account for 80 % of total microbial biomass in all groups. Cyanobacteria were found most abundantly in limestone wall instead of water or soil of weathering limestone. The PCoA dimensional patterns of each phylum showed a trace of microbial community dynamic, which might be affected by environmental factors. This study provided the insights to understand how environmental factors worked together with microbial community to shape landscape structures.

FEMS7-1074
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SURVEILLANCE AND EVALUATION OF THE INFECTION RISK OF ACANTHAMOEBA IN VARIOUS AQUATIC ENVIRONMENTS

B.M. Hsu[1]
[1]*National Chung Cheng University, Earth and environmental sciences, Chiayi, Taiwan R.O.C.*

Backgrounds

Acanthamoeba is one kind of free-living amoebae (FLA) which ubiquitous in various aquatic environments. Several *Acanthamoeba* species are pathogenic and host other pathogens such as *Legionella*, but the presence of *Acanthamoeba* and its parasites as well as the related infection risk are not well known.

Objectives

In this study, the surveillance and evaluation of the infection risk of *Acanthamoeba* in different aquatic environments was investigated. Water samples were collected from a river, intake areas of drinking water treatment plants, and recreational hot spring complexes in Taiwan.

Methods

A total of 140 water samples were tested for the presence of *Acanthamoeba* spp.. In addition, phylogenetic characteristics and water quality parameters were also assessed.

Conclusions

The pathogenic genotypes of *Acanthamoeba* T4 were abundant in the hot spring water. Taken together, *Acanthamoeba* contamination in recreational hot springs and drinking water source warrants more attention on potential legionellosis and amoebae infections.

FEMS7-1547
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INTESTINAL MICROBIOTA AND MICRONUTRIENT DEFICIENCIES OF CAMBODIAN CHILDREN

C. Humblot[1], V. Greffeuille[1], M. Parker[2], W. Turpin[3,4], K. Burja[5], P. Picq[1], J. Berger[1], C. Chamnan[6], K. Kuong[6], M. Sinuon[7], S. Fortin[1], F. Wieringa[1]
[1]Institut de Recherche pour le Developpement, Nutripass, Montpellier, France
[2]PATH-USA, Program for Appropriate Technology in Health, Seattle, USA
[3]University of Toronto, Medical Sciences, Toronto, Canada
[4]Mount Sinai Hospital, Zane Cohen Centre for Digestive Diseases, Toronto, Canada
[5]United Nations World Food Programme, WFP, Phnom Penh, Cambodia
[6]Ministry of Agriculture- Forestry and Fisheries, Department of Fisheries Post-Harvest Technologies and Quality Control, Phnom Penh, Cambodia
[7]Ministry of Health, National Centre for Malaria and parasites, Phnom Penh, Cambodia

Backgrounds

Intestinal microbiota has been extensively studied for many years, with associations between alterations in microbiota composition and several diseases firmly established. Surprisingly few data exist on associations between microbiota composition and micronutrient status. Also, most studies have been done in adults or infants, but rarely in schoolchildren. In Cambodia, malnutrition and micronutrient deficiencies are still common. We analysed data from a large intervention trial in Cambodian schoolchildren (FORISCA) for associations between microbiota and micronutrient status.

Objectives

The primary objectives of this study are to characterize the intestinal microbiota of Cambodian schoolchildren and possible associations with (micro)nutrient status. Secondary objectives include associations of microbiota composition with systemic inflammation, parasite infestation and scores on cognitive tests. Stability of microbiota along time has also been estimated.

Methods

In total, data was available on 450 schoolchildren (6 to 16 years old) from 16 schools at baseline and after 6 months of consumption of micronutrient-fortified rice (or placebo). Nutritional status indicators included anthropometry and biomarkers for iron, zinc and vitamin A status. Illumina sequencing of PCR amplicons of 16S rRNA coding genes have been performed on DNA extracted from stool samples.

Conclusions

Preliminary results suggest that microbiota composition is related to biomarkers of micronutrient status and cognitive test scores. Intestinal microbiota composition seems to evolve along time.

FEMS7-2497
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

IDENTIFICATION OF REPRODUCTIVE MANIPULATOR BACTERIAL SPECIES IN PEST MITES OF ECONOMIC IMPORTANCE FOR CITRUS IN SPAIN

T. Pina[1], C. Marc[1], J. Cruz[1], B. Sabater-Muñoz[2], J. Josep[1], M. Hurtado Ruiz[1]
[1]Universitat Jaume I, Ciències Agraries i del Medi Natural, Castellon de la Plana, Spain
[2]Trinity College University of Dublin, Systems Biology and Abiotic Stress, Dublin, Ireland

Backgrounds

Citrus is an economic important crop threatened by several pests. Among them, *Tetranychus urticae* and *Panonychus citri* (Acari: Tetranychidae) are of special consideration. During the last years, our group has studied the genetic structure of *T. urticae* finding a relationship between genotypes and pesticides applications in citrus orchards. In addition, a sub-division of *T. urticae* populations was depicted between the main crop and the ground cover, highlighting the putative presence of plant-specialist *T. urticae* strains. Tetranychidae mites have been characterized for the presence of reproductive manipulator endosymbionts, like *Wolbachia*, *Cardinium*, *Rickettsia* and *Spiroplasma*. One of this species, *Wolbachia*, has been implicated in pesticide resistance of mites, being also of consideration to biotechnological control approaches (para-transgenesis). However, the distribution of these bacterial species and its relationship with mite population's genetic structure still deserves studies.

Objectives

Our main objective is to determine the presence of these four species in Spanish Tetranychidae populations on citrus orchards

Methods

After external surface sterilization, mite samples were screened by PCR with universal 16S primers, *Wolbachia* (wsp, fstZ in addition to 16S), *Cardinium*, *Rickettsia* and *Spiroplasma* specific primers. Samples were scored as positive in bacterial symbionts other than *Wolbachia*, *Cardinium*, *Rickettsia* or *Spiroplasma* if only amplified with universal 16S primers. PCR fragments were sequenced and aligned against Gene bank sequences of reference

Conclusions

Spanish Tetranychidae populations showed a differential *Wolbachia* infection level, with some populations showing additional bacterial species. The relationship between *Wolbachia* presence and host plant specialization of citrus mite populations deserves further research.

FEMS7-2508
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

METAGENOMIC CHARACTERIZATION OF MICROBIOTA IN ECONOMIC IMPORTANT MITES OF CITRUS

T. Pina[1], B. Sabater-Muñoz[2], J. Jaques[1], M. Hurtado Ruiz[1]
[1]Universitat Jaume I, Ciències Agraries i del Medi Natural, Castellon de la Plana, Spain
[2]Trinity College University of Dublin, Systems Biology and Abiotic Stress, Dublin, Ireland

Backgrounds

Plant-organism interactions have been usually seen as a two component problem only. Over the few past years, bacterial endosymbionts of arthropods have been incorporated as a third part of this plant-arthropod interaction. The family Tetranychidae (Acari) comprises worldwide citrus pests as *Tetranychus urticae*. Due to its impact, interaction studies were started finding that plant defense response induced by *T. urticae* depended on citrus rootstock and/or cultivar. The implication of bacteria from the Acari microbiota in this plant-arthropod interaction still deserves research

Objectives

To ascertain the partners in this plant defense response variation, our objective is to determine the microbiota composition of different *T. urticae* populations, feeding on two citrus species used as main rootstocks of Clementine mandarins, and of other Acari of economic importance in citrus

Methods

Mites were sampled in citrus fields taking into consideration rootstock and variety. In addition, a *T. urticae* laboratory strain and its tetracycline-treated sister strain were also tested. After external surface sterilization, mite samples were subjected to total DNA extraction using Zymobiomics kit (Zymo) or by a Salting-out ('in house' protocol). The v3-v4 region of 16S rDNA was deep sequenced with MiSeq (Illumina) at 2x300bp cycle. Fastq files were processed and OTUs identified to determine bacterial diversity indexes. Finally, bacterial species were identified by blast homology

Conclusions

Mite populations in Spanish citrus present a complex microbiota. *Wolbachia* has been found mainly in *T. urticae* samples. Identified microbial assemblages are discussed by their implication in citrus-Acari interaction focused on biological control programs

FEMS7-1430
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BACTERIAL LEACHING OF MINERALS USING STREPTOMYCES

J. Hurtado[1], *D. Ugarte*[1]
[1]*Universidad Peruana Cayetano Heredia, Ciencias celulares y moleculares, Lima, Peru*

Backgrounds

Microorganism play a key role in arsenopyrite oxidation in natural and commercial processes. There are many organisms involved in the bacterial leaching operations. It has been shown the presence of actinobacteria in leaching enviroments.

Objectives

Demonstrate the use of Streptomyces in the bacterial leaching of arsenopyrite

Methods

Ten isolates of Streptomyces from arsenopiryrite, pyrite, polimetalic sulfides and magnetite from Peruvian mining zones have been characterized and they have been tested by growth on arsenopyrite tailings. Only two isolates were able to develop with this mineral. Streptomyces sp. E1 and Streptomyces variabilis AB5 leached 19.1 % and 15.5 % of arsenic present in tailings while, the control without inocula, only showed 2.5% of leaching.

Conclusions

This study demonstrated that Streptomyces is able to degradate arsenopyrite

FEMS7-0254
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

FLAVONOIDS AND IAA CROSSTALK: COLONIZATION OF MAIZE ROOT BY ENDOPHYTIC FUNGI FUSARIUM CULMORUM SP. PZ

A. Hussain[1], *A. Mehmood*[1], *M. Irshad*[1]
[1]*Abdul Wali Khan University, Botany, Mardan, Pakistan*

Backgrounds

Molecular crosstalk between microbes and their host is the first step toward the establishment of any association. Plant roots release a variety of signal molecules to differentially treat the beneficial and harmful microbes.

Objectives

Current study focuses on the role of flavonoids and IAA as signals for molecular dialogue between endophytic fungus and maize root.

Methods

Endophytic fungi *Fusarium culmorum* sp. Pz was isolated from the roots of drought stressed *Asphodelus tenifolium* roots. Identity of the strain was confirmed by homology of the ITS region of 18 S rDNA sequence. Flavonoids and IAA were analyzed in fungal culture filtrate and exudate of maize root (with and without endophyte) by LC-ESI-MS/MS.

Conclusions

The strain was able to produce a number of phytostimulants and signaling compounds including indole-3-acetic acid (IAA), flavonoids and sugar. Its culture filtrate contained 33.2±0.8, 275.1±8.7 and 186.6±15.7 µg/mL of IAA, total flavonoids and sugar respectively. The strain effectively colonized in the roots of maize and subsequently enhanced growth of its host. Inhibition of flavonoids or IAA exudation by maize root effectively reduced colonization of the endophyte in maize root to 89% of the control. Similarly, colonization of root by endophyte with repressed flavonoids was reduced 62% of the control suggesting a flavonoids talk between the two partners. Suppression of IAA biosynthesis in the endophyte drastically affected its colonization in the maize root. It is concluded that a molecular crosstalk of maize roots and endophytic *Fusarium culmorum* sp. Pz is necessary for subsequent endophytic association between them.

FEMS7-2099
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INDUSTRIAL COATING SYSTEMS ARE A SPECIAL NICHE FOR BURKHOLDERIA STRAINS JEOPARDIZING CAR LACQUER PROCESSES

K. Huth-Herms[1], A. Sisman[1], J. Erhardt[1], A. Spielvogel[1], D. Oberschmidt[1], E. Uhlmann[1]
[1]Fraunhofer Institute for Production Systems and Design Technology, Microproduction technology, Berlin, Germany

Backgrounds

Microbial contaminations of water-based varnishes are a threat in industrial coating systems. Microorganisms of the genus *Burkholderia* are frequently carried over from pretreatment steps causing coating defects on surfaces during the final coating step. Despite regular and increased addition of biocides, microbial contamination leads to production downtime and financial losses.

Objectives

Four strains from the pretreatment (PT1, PT2) and coating (CT1, CT2) area were isolated. They were analyzed and compared to *Burkholderia cepacia* (DSM7288) regarding their growth behavior in different cultural and biocide conditions. All strains show an improved growth in minimal culture compared to carbon rich culture, with generation rates between 42 and 60 minutes. In addition, under such growth conditions the isolates from the coating systems formed significantly stronger biofilms than DSM7288. Interestingly, upon the addition of biocide the strain CT1 forms biofilms very fast and survives biocide concentration normally used in industrial settings.
The typical self-aggregation mechanism of *Burkholderia* could be confirmed for all isolated strains. Interestingly, under moving conditions the cells formed aggregates after only one hour of cultivation.

Methods

Microbial growth was examined by determining the optical density. Biofilmformation was detected by crystal violet staining. Aggregate formations was identified by microscopy and agar plate cultivation.

Conclusions

It is assumed that the aggregation mechanism is responsible for biofilm formation on the surface of coatings solids and not on the surface of the coating vessel. *Burkholderia* strains possess a fast adaptation system in order to survive harsh environmental conditions. Future work will aim to understand the genetic background of these changes.

FEMS7-0546
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SEARCHING AND COMPARATIVE ANALYSIS OF PUTATIVE BACTERIOCIN-LIKE GENE LOCI IN THE GENOMES OF S. PNEUMONIAE AND ITS CLOSE RELATIVES

L. Ikryannikova[1], M. Malakhova[1], G. Lominadze[2], I. Karpova[3], E. Kostryukova[3], N. Mayansky[2], A. Kruglov[4], E. Klimova[5], E. Ilina[1], V. Govorun[6]
[1]Federal Research and Clinical Center of Physical-Chemical Medicine, Laboratory of the Molecular Genetics of Microorganisms, Moscow, Russia
[2]Federal State Budgetary Inst. "Scientific Center of Children Health" of RAMS, Federal State Budgetary Inst. "Scientific Center of Children Health" of RAMS, Moscow, Russia
[3]Federal Research and Clinical Center of Physical-Chemical Medicine, Laboratory of the post-genome investigations, Moscow, Russia
[4]National Agency for Clinical Pharmacology and Pharmacy, Laboratory of Microbioilogy, Moscow, Russia
[5]A.I. Evdokimov Moscow State University of Medicine and Dentistry, A.I. Evdokimov Moscow State University of Medicine and Dentistry, Moscow, Russia
[6]Federal Research and Clinical Center of Physical-Chemical Medicine, Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russia

Backgrounds

Bacterial competition between members of microbiome is often mediated by bacteriocins – small peptides produced by microorganisms which are capable to kill competitor strains or species. Bacteriocinogeny, i.e. an ability to produce bacteriocins, can give an evolutionary advantage to the strain-producer.

Objectives

In this work, we studied bacteriocin production activity of some species (*S. pneumoniae*, *S. pseudopneumoniae* and *S. mitis*) inside the mitis group of viridans group streptococci (VGS), which are frequent colonizers of the human nasopharynx. To estimate a bacteriocinogeny potential, we applied traditional culture-based methods as well as *in silico* searching of the bacteriocin-like peptides encoding genes across the genomes of the study strains.

Methods

Nine strains of VGS were inspected for the bacteriocin production by the deferred antagonism bacteriocin assay using *Staphylococcaciae* strains as indicators. A set of genes encoding potential bacteriocins was outlined by using of the web-resource BAGEL3 (http://bagel.molgenrug.nl/index.php/bagel3).

Conclusions

All strains under study were found to be active against *S. aureus, S. epidermidis* and *S. haemolyticus*. A thorough study of genomes showed that the ways of bacteriocin production by pneumococci might be more complicated than it was usually considered, due to the presence of some more putative bacteriocin-associated gene clusters in their genomes. However, we could not explain a high inhibition activity of at least one of three *S. mitis* strains based on the investigation of its genome.

This work was supported by the Russian Science Foundation (RSF) № 15-15-00158 grant.

FEMS7-2765
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ISOLATION OF NITROGEN FIXING BACTERIA ISOLATED FROM SOIL SAMPLES IN SOUTH KOREA AND CHARACTERIZATION OF ITS PLANT GROWTH PROMOTION

W.T. Im[1], *C. Gyumiin*[1]
[1]*National Hankyong University, Biotechnology, Anseong-si, Republic of Korea*

Backgrounds

Nitrogen (N) is an essential element for all living organisms, being a constituent of proteins, nucleic acids, and many other biomolecules. The largest pool of nitrogen in the biosphere is atmospheric dinitrogen (N_2). The input of N_2 into the biogeochemical nitrogen cycle occurs by reduction of N_2 to two ammonia molecules (NH_3) by nitrogen fixing bacteria.

Objectives

This study focused on the isolation and characterization of nitrogen fixing bacteria from the plant rhizosphere of various soil samples in Anseong and Pocheon province, South Korea, and investigate their effects on plant growth.

Methods

50 strains were isolated using nitrogen free agar from paddy soil and ginseng cultivating soil and were identified 16S rRNA gene sequencing. Two plant growth promoting, nitrogen-fixing bacterial strains, *Mesorhizobium plurifarium* EMML 409^T and *Burkholderia choica* Gsoil 652, were selected based on their growth curves in seven different media.

Conclusions

Their plant promoting abilities in the early growth phase were studied in cabbage (*Brassica campestris* subsp. *napus*). When the plant seedlings were treated with these strains, their fresh weight, dry weight and root length were increased.

FEMS7-1999
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COLLATERAL SENSITIVITY NETWORKS ENABLE TARGETED THERAPEUTIC STRATEGIES AGAINST DRUG RESISTANCE IN PSEUDOMONAS AERUGINOSA

L. Imamovic[1], M. Hashim Ellabaan[1], M. Ana[1], S.M. Søren Molin[1], H.K.J. Helle Krogh Johansen[1], S. Morten[1]
[1]Denmark, Technical Universisty of Denmark, Lyngby, Denmark

Backgrounds

Development of drug resistance in a serious challenge for success of drug therapies. Treatment strategies incorporating critical vulnerabilities induced by antimicrobial resistance development could have a great utility in clinical treatments. Previously, we and others reported the vulnerabilities of drug resistant cells (collateral sensitivities) of Escherichia coli. Applying same principles in a major cystic fibrosis pathogen, Pseudomonas aeruginosa, could significantly improve the treatment outcome.

Objectives

The aim of this study is to evaluate systematically phenotypic and genotypic adaptive changes that emerge as a consequence of drug resistance evolution in P. aeruginosa.

Methods

We performed experimental adaptive evolution in media that resemble the chemical composition of CF lungs for PAO1 and DK2 strains. Antibiotic resistant strains were selected during 10-day passage supplemented with clinically relevant antibiotics. To underline the genetic basis for collateral sensitivity and resistance, genomes of resistant strain were sequenced on a MiSeq platform.

Conclusions

Drug resistance development limits the successful application of many available anti-pseudomonal drugs. Studying drug resistance evolution may thus hold the key to understanding how the current therapeutics may change upon subsequent side effects. The drug deployment based on collateral sensitivities might integrate several factors important in the treatment of CF, such as 1) presence of heterogeneous population, 2) immunomodulatory properties 3) presence of other pathogens. Furthermore, identifying the genetic link to drug resistance has a high relevance in treatment of chronic infections.

FEMS7-0388
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CHARACTERISATION OF TWO NEW SOIL-DWELLING PSEUDOMONAS ISOLATES ABLE TO BREAK-DOWN AIR POLLUTANTS

V. Imperato[1], S. Thijs[1], Ł. Kowalkowski[2], Y. Douwen[1], R. Carleer[1], J. Van Hamme[3], S. Gawronski[2], N. Weyens[1], J. Vangronsveld[1]
[1]Hasselt University, Centre for Environmental Sciences, Diepenbeek, Belgium
[2]Warsaw University, Department of Life Sciences, Warsaw, Poland
[3]Thompson Rivers University, Department of Biology, Kamloops, Canada

Backgrounds

Air pollution is considered as the world's largest single environmental health threat. It is clear that more effective pollution control measures need to be taken. Since airborne contaminants are regularly carried by rain into the soil, we hypothesise that soil microorganisms might play a significant role in an effective air pollution control.

Objectives

We tested soil bacteria for their ability to degrade common airborne pollutants.

Methods

We sampled soil in the world's first oil field, Bóbrka (Poland). For the *in situ* cultivation, we used innovative miniature diffusion chambers, each approximately inoculated with a single environmental cell. Consecutively bacteria were purified, tested for diesel-degradation, toluene degradation with GC-MS, and ability to produce VOCs for plant growth promotion. Presence and expression of catabolic genes (oxygenases) directly involved in the PAHs degradation pathway were evaluated with degenerate primers, and followed by draft genome sequencing.

Conclusions

Soil bacteria of diverse phylotypes were isolated for their high potential to degrade diesel. We found 14 new *Pseudomonas* sp. carrying naphthalene dioxygenase genes, 2 of them were selected for whole genome sequencing. Preliminary data on GC-MS showed a disappearance of 40 µg L^{-1} toluene from the air phase in 5 days. Further genome-mining will elucidate which other pathways are involved to degrade toluene and related PAHs.
Bacteria dwelling in heavily contaminated soils appear to be able to degrade airborne pollutants. Two of the isolated strains will be further tested in controlled cuvette-experiments to explore their potential to mitigate air-pollution and improve environmental quality in function of human health.

FEMS7-0348
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BIOLOGICAL NITRIFICATION INHIBITION BY RICE ROOT EXUDATES IN TWO DIFFERENT SOILS OF URUGUAY

G. Illarze[1], *P. Irisarri*[1]
[1]*Facultad de Agronomía, Plant Biology, Montevideo, Uruguay*

Backgrounds

Rice crop is an important source of greenhouse gases, mostly CH_4, but also of N_2O. Nitrous oxide in soils is originated mainly by the microbial processes of denitrification and nitrification. Recently the first evidence of biological nitrification inhibition (BNI) by root exudates was reported and it varied with the plant cultivar.

Objectives

The objective of this work was to check and compare the BNI potential of the roopt exudates of two rice cultivars, El Paso 144 and INIA Tacuarí, and to evaluate their inhibitory effect on the activity and abundance of bacteria and archeae NH_4^+ oxidizers.

Methods

This effect was tested in two soils that differed in their organic matter content, among other characteristics.With the bioassay using a *Nitrosomonas europea* recombinant strain, the BNI effect of both cultivars was verified. The cultivar Tacuarí presented higher BNI and in the microcosm assay the BNI effect was higher than the produced by the synthetic nitrification inhibitor DCD, in the soil with lower organic matter content. The *amo*A copy number of archeae, quantified with qPCR, was not affected by the root exudates addition, while that of bacteria decreased.

Conclusions

These rice cultivars BNI activities should be assayed in the field as a N_2O mitigation strategy.

FEMS7-1541
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ISOLATION AND CHARACTERIZATION OF ANTAGONISTIC BACILLUS STRAINS FROM THE RHIZOSHPERE OF TOMATO AS POTENTIAL BIOCONTROL AGENT AGAINST FUSARIUM OXYSPORUM F. SP. LYCOPERSICI

M. Jangra[1], *S. Sharma*[1], *S. Sharma*[2]
[1]*Indian Institute of Technology-Delhi, Centre for Rural Development and Technology, New Delhi, India*
[2]*Indian Institute of Technology-Delhi, Department of Biochemical Engineering and Biotechnology, New Delhi, India*

Backgrounds

*Fusarium*wilt is one of the most important diseases of tomato. In India, it causes 45% of yield losses. Its current control includes use of chemical fungicides. These chemicals have harmful effects on the environment and human health. As an alternative different biocontrol agents, can be used which is *Bacillus* that is known to have antagonistic activity against various plant pathogens.

Objectives

The objective of the present work was to isolate and characterize *Bacillus* strains from tomato rhizosphere with antagonistic activity against *Fusarium oxysporum* f. sp. *lycopersici* for application as biopesticides.

Methods

A collection of 49 *Bacillus* isolates were obtained from the rhizosphere of the unhealthy tomato plant on the basis of morphology on Hichrome Bacillus agar. Dual plate assay was used to observe the efficacy of all the isolates against pathogen. Antifungal properties like production of biofilm, hydrolytic enzymes, ammonia and hydrogen cyanide were studied.

Conclusions

One isolate was selected on the basis of analysis of the antifungal compounds involved in their antagonistic activity. It exhibited deleterious effects like production of protease, ammonia and hydrogen cyanide. This isolate was capable of producing indole acetic acid (IAA), a plant growth promoting trait and possesses higher ability for biofilm formation (35%) in microtitre plate assay. The isolate was identified as *Bacillus cereus* based on the analysis of the 16S rRNA gene sequence. Since, *Bacillus* isolates exhibited several traits beneficial to the host; they may be used to develop new safer and effective formulations as an alternative to chemical fungicides.

FEMS7-0667
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EVALUATION OF SUITABILITY AND SAFETY IN COMMERCIAL PROBIOTICS FOR ANIMAL FEEDING

S.E. Jeong[1], C. Byung Hee[1], K. Hye Rim[1], J. Che Ok[1]
[1]Chung-Ang University, Life Science, Seoul, Republic of Korea

Backgrounds

Because the wide use of antibiotics as animal feed additives leads to more concerns about widespread of antibiotic resistance genes, the use of antibiotics as growth promoters was prohibited in Korea. A variety of commercial probiotics using microorganisms have been developed as alternatives of antibiotics. However, product description about microbiological compositions is not often in accordance with their contents in products. In addition, pathogen contamination in commercial probiotics also have not been extensively investigated.

Objectives

The aim of this work is to verify the suitability and safety of commercial probiotics produced in Korea as animal feeding.

Methods

Bacterial 16S rRNA genes of fifty commercial products of twenty-six brands were amplified and sequenced using 454 high-throughput pyrosequencing and raw data generated were processed and classified using RDPipeline (http://pyro.cme.msu.edu). Potentially pathogenic bacterial sequences from the raw data were found by BLASTN searches using 16S rRNA gene sequences of pathogens that are toxic to human and livestock (chicken, cattle, and pig) derived from the PHI (Pathogen Host Interaction) database.

Conclusions

Bacterial community analysis indicated that bacterial compositions of some probiotic products were similar with labeled information; however, some differences were also observed in other products. Potentially pathogenic sequences classified as *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, and *Escherichia coli* at a 97% sequence similarity criterion were approximately 2% of the total sequencing reads. The *regA*, toxin A regulatory gene, of *P. aeruginosa* was detected from some commercial products by PCR. Thus, strict regulation and quality control in the production of probiotics as commercial products may be necessary.

FEMS7-2087
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SCREENING AND IDENTIFICATION OF HIGHLY EFFICIENT NITROGEN FIXING BACTERIA FROM AGRICULTURAL LAND SOILS TO UTILIZE AS BIO-FERTILIZER

S.H. Jeong[1], J. Kim[1], S.S. Lee[1]
[1]Kyonggi University, Life science, Suwon, Republic of Korea

Backgrounds

Fertilization of agricultural area is required for improving of yield of the crop by using microbial nitrogen metabolism.

Objectives

This study is to screen and identify the highly efficient free-living nitrogen fixing bacteria *Cedecea* sp. MK7 from agricultural soils. Moreover, *Cedecea* sp. MK7 improves the growth and yield of the plant.

Methods

Soil sampling was performed from Mun-kyeong and Yeon-cheon, South Korea. Ashby's media were used for isolation of nitrogen fixing bacteria. Isolates were analyzed to confirm nitrogen fixation activity by acetylene reduction assay (ARA). Among the nitrogen fixation bacteria, high efficient bacteria *Cedecea* sp. MK7 applied to perennial ryegrass pots for evaluating the plant growth. For the control, only water was applied to the plant. After growing plant, dry weight of plant and length of shoot was determined between experimental group and control group. Dry weight of the experimental group was heavier three-fold in response to control pot experiment. The length of the stem was 1.4-fold longer than the control.

Conclusions

From acquisition results, we confirmed the effects of nitrogen fixation bacteria on plants. Furthermore, we will study auxin, siderophore producing ability and phosphate solubilizing activity of bacteria. Nitrogen fixation bacteria can contribute on the agricultural industry.

FEMS7-2465
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

IDENTITY OF MICROORGANISMS RESPONSIBLE FOR FOAMING IN ANAEROBIC DIGESTERS FOR BIOGAS PRODUCTION

C. Jiang[1], S.J. McIlroy[1], L. Hao[1], B. McIlroy[1], M. Nierychlo[1], P.H. Nielsen[1]
[1]Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg, Denmark

Backgrounds

Anaerobic digesters (ADs) are commonly implemented at wastewater treatment plants for the conversion of surplus sludge to biogas. Foaming is a major operational problem in these systems where hydrophobic microbial cells are thought to stabilize foams. It is still not clear which organisms are specifically responsible for such events. It may be bacteria coming with the surplus sludges, e.g. *Microthrix* or *Gordonia*, or some that actively grow in the ADs.

Objectives

The objective of this study was to identify putative foaming-forming microorganisms in ADs.

Methods

Amplicon sequencing targeting the 16S rRNA gene was applied for analyses of the microbial communities of full-scale AD plants with and without foaming and, where possible, compare the communities of the bulk sludge and the foaming layer. Fluorescence *in situ* hybridization (FISH) was applied to further investigate putative foam formers.

Conclusions

Identification of potential foam forming organisms based on surveys was difficult attributed to the complexity of these systems. A focus on foaming incidents in individual plants was more successful. During a foaming episode at one plant, the bacterial community structure showed substantial variation between the top foaming layer and digester sample. Members of the A6 phylotype (phylum Chloroflexi), a novel phylotype classified to the family Ruminococcaceae (Firmicutes), and vadinBC27 wastewater-sludge group (Bacteroidetes) displayed relatively higher abundances in top foaming layer, which was confirmed with FISH for the former two. Members of the A6 were abundant in other plants with foaming issues and may be key organisms responsible for foaming in Danish systems.

FEMS7-1479
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

A NOVEL ARSENATE RESPIRING BACTERIUM ALKALIPHILUS SP. IMB: ISOLATION, CHARACTERIZATION, AND FUNCTION

C. jing[1]
[1]*Research Center for Eco−Environmental Sciences- Chinese Academy of Sciences, State Key Laboratory of Environmental Chemistry and Ecotoxicology, Beijing, China*

Backgrounds

Arsenic (As) redox transformation mediated by As(V)-respiring microorganisms plays an important role in regulating As speciation and mobility in the environment. The importance of As(V)-respiring bacteria in As geochemical cycling in the Hetao Plain, a typical area with geogenic As in China, motivated our study.

Objectives

The objectives of this study are 1) to isolate and characterize an As(V)-respiring bacterium in the Hetao Plain, and 2) to explore the As(V) respiration mechanism and effect of the isolate.

Methods

The soil sample was collected in the Hetao Plain, China. Standard protocols were followed to isolate and characterize the As(V)-respiring bacterium including TEM and synchrotron-based STXM. Batch incubation and flow-through column experiments were used to explore the effect of the isolate on the As mobility.

Conclusions

A novel As(V)-respiring bacterium, *Alkaliphilus* sp. IMB, was isolated from soil in the Hetao Plain, China. Strain IMB is Gram-positive, spore-forming, and rod-shaped. High similarity of 16S rRNA gene sequence was found between IMB and *Alkaliphilus oremlandii* strain OhILAs, with DNA-DNA relatedness of 44.3%. The 16S rRNA gene sequence and DNA-DNA hybridization results showed that the isolate was a new strain of the genus *Alkaliphilus*. A partial *arrA* gene sequence was amplified. An assay for As(V) reductase activity and STXM results showed that strain IMB could reduce As(V) to As(III) by a respiratory reductase mostly located at the outside of the cell membrane. Our batch and column experiments highlighted the importance of IMH in liberating As from a As-laden industrial solid waste.

FEMS7-1224
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MULTIOMIC APROACH OF FUNCTIONAL AND ECOLOGICAL NETWORKS INTO THE HIV GUT-ASSOCIATED DYSBIOSIS

J.F. Vazquez Castellanos[1], S. Serrano Villar[2], M. Ferrer[3], M.D. Soto del Rio[4], N. Jimenez Hernández[1], S. Gayo[1], S. Moreno[2], A. Latorre[1], A. Moya[1], G. Maria Jose[1]
[1]Unidad Mixta de Investigacioón en Genómica y Salud de la Fundación para el Fomento de la Investi gación Sanitaria y Biomédica de la Comunidad Valenciana FISABIO-
Salud Pública y el Instituto Cavanilles de Biodiversidad y Biología Evolutiva Universidad de Valencia, Genómica y salud, Valencia, Spain
[2]Hospital Universitario Ramón y Cajal- Facultad de Medicina- Universidad de Alcala IRYCIS, Department of Infectious Diseases, Madrid, Spain
[3]Institute of Catalysis- Consejo Superior de Investigaciones Científicas CSIC, Biotecnología de Sistemas, Madrid, Spain
[4]Università degli Studi di Torino, Department of Agricultural- Forest and Food Sciences, Turin, Italy

Backgrounds

The HIV infection is characterized by an early depletion of the mucosal associated lymphocytes Th17 and a disruption of the mucosal integrity. The HIV-gut associated bacterial community is significantly different from the healthy subjects showing an altered structure with an increment of gram-negative bacteria and a dysbiotic metabolism. The changes into the microbiota composition are related with the HIV inflammation

Objectives

The aim of the study is to integrate different “omics” data, retrieved from a cohort of HIV+ infected subjects, to describe in a holistic manner the HIV-gut associated community applying a multivariate statistical analysis.

Methods

Clinical data as well as metagenomes, metatranscriptomes and metabolomes from fecal samples were retrieved from a cohort of 54 HIV+/Control subjects. Ecological networks were estimated by means of the “sparcc” software. Functional networks were estimated using the R package “KEGG graph. Finally a Bayesian network from all the “omic” data was created (R package bnlearn), using as seed the results retrieved form the generalized linear models (R package“glmnet”). All the plots and network statistics were calculated using the R package Igraph.

Conclusions

The Bayesian modeling showed that the disruption of the mucosal associated lymphocytes might lead to an establishment of a dysbiotic microbiota that is functionally adapted to oxidative stress. The decay of the expression of genes related to the butyrate production and the proliferation of gram-negative bacteria were associated to inflammation parameters. Finally, species and pathways that are statistically increased into the HIV+ bacterial communities resulted into important hubs that maintain the stability of the dysbiotic community.

FEMS7-0468
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

METAGENOMICS REVEALS ANTHROPOGENIC IMPACT ON BACTERIAL DIVERSITY OF WESTERN BALKANS GLACIAL LAKES SEDIMENTS

M. Malešević[1], N. Mirković[1], J. Lozo[2], J. Begović[1], K. Novović[1], B. Filipić[3], G. Vukotić[2], M. Miljković[1], M. Kojić[1], B. Jovčić[2]
[1]Institute of Molecular Genetics and Genetic Engineering, Laboratory for Molecular Microbiology, Belgrade, Serbia
[2]Faculty of Biology - University of Belgrade, Chair of Biochemistry and Molecular Biology, Belgrade, Serbia
[3]Faculty of Pharmacy, Department of Microbiology and Immunology, Belgrade, Serbia

Backgrounds

Potential vanishing of Balkans glaciers due to climate change could endanger existing glacial lakes, which points the need for conservation and biodiversity studies of this ecological niche. It is well recognized that unexplored ecological niches and their microbial communities metabolic potential could be a gold mine of secondary metabolites with importance for biotechnology, medicine and pharmacy.

Objectives

Our primary goal was to assess the biodiversity of bacterial communities of sediments from selected Western Balkans glacial lakes and to reveal the anthropogenic impact on those communities.

Methods

Sediment samples were collected from three glacial lakes the Plav lake (located in town of Plav, high anthropogenic activity), the Black lake (recreational site, medium anthropogenic activity) and the Donje Bare lake (remote lake with minimal anthropogenic activity). Chemical composition of sediments was determined by standard chemical procedures. DNA extraction from each sediment sample was performed using a MoBio UltraCleanTMSoil DNA isolation kit. 16S rDNA metagenome libraries were generated with the 454 Life Sciences GS-FLX Titanium platform.

Conclusions

Organic compounds that are product of anthropogenic activities were most abundant in Plav lake. A number of bacteria from families and genera that could be coupled to anthropogenic impact were also found only in the sediment from the Plav lake, or had the greatest representation comparing to other sediments. Prevalence of bacteria that are typical for low human impacted area was the highest in Donje Bare lake. Anthropogenic impact was shown to be important for shaping the sediment communities, beside the biological and biogeochemical factors.

FEMS7-2094
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EVOLUTION AND STABILITY OF COOPERATION IN GROWING POPULATIONS OF PSEUDOMONAS PUTIDA KT2440

H. Jung[1], B. Felix[1], W. Karl[2], L. Matthias[2], F. Erwin[2]
[1]Ludwig-Maximilians-University, Microbiology, Martinsried, Germany
[2]Ludwig-Maximilians-University, Statistical and Biological Physics, Munich, Germany

Backgrounds

The development of bacterial populations crucially depends on social interactions between cells such as competition and cooperation (West et al. (2007) *Annu. Rev. Ecol. Evol. Syst.* **38**, 53). For example, production of a public good by a cooperator can support growth of a population. However, non-producing individuals that benefit from the public good without paying the costs for its production have a selection advantage over the cooperator. As a consequence, the cooperator frequency may decline, and the entire population may destabilize.

Objectives

We set out to predict and experimentally test conditions leading to an increase in cooperator frequency in bacterial metapopulations growing in liquid culture.

Methods

Experiments were performed with two strains derived from *Pseudomonas putida* KT2440: a constitutive producer of the iron-chelating siderophore pyoverdine and a corresponding non-producer. The rate and the metabolic costs of pyoverdine production as well as the benefit of pyoverdine for growth under iron limiting conditions were experimentally determined. The parameters were used to formulate a mathematical model that predicts the growth dynamics of metapopulations consisting of groups with a random distribution of initial producer frequencies.

Conclusions

Both mathematical model and experiment agree well in demonstrating a transient increase of the global producer frequency due to competing inter- and intra-group processes during growth. Our results show that cooperation can evolve in a native microbial system due to demographic noise across a metapopulation.

FEMS7-0647
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ISOLATION AND CHARACTERIZATION OF CONFLUENTIMICROBIUM NAPHTHALENIVORANS NS6T, A NAPHTHALENE-DEGRADING BACTERIUM

H.I. Jeong[1], H.S. Jung[1], K.H. Kim[1], S.E. Jeong[1], C.O. Jeon[1]
[1]Chung-Ang University, lifescience, Seoul, Republic of Korea

Backgrounds

The investigation of bacteria responsible for biodegradation of polycyclic aromatic hydrocarbons (PAH) is of great interest in a sea-tidal flat contaminated with crude oil.

Objectives

The purpose of this study was to isolate and characterize bacteria responsible for PAH degradation from a contaminated tidal flat.

Methods

A slurry type enrichment system with naphthalene as a sole carbon and energy source was established for the isolation of PAH-degrading bacteria.

Conclusions

A new naphthalene-degrading bacterium, designated *C. naphthalenivorans* NS6^T, was isolated from sea-tidal flat sediment contaminated with crude oil. Degradation tests revealed that strain NS6^T had a great degradation ability for naphthalene in tidal flat sediment. In addition, the degradation tests using ^{12}C- and ^{13}C-naphthalene showed that salicylate, gentisate, and $^{13}CO_2$ were detected as metabolites or products of strain NS6^T, suggesting that strain NS6^T mineralizes naphthalene to $^{13}CO_2$ completely via the gentisate pathway. The genome of strain NS6 was completely sequenced for the better understanding of its naphthalene biodegradation properties, indicating that the genome consisted of a circular chromosome and three plasmids. We found that a plasmid, pNS6002, contains a gene cluster without salicylate 5-hydroxylase probably involved in naphthalene degradation although strain NS6^T metabolizes naphthalene via the gentisate pathway, suggesting that strain NS6^T may convert salicylate to gentisate via a different enzyme system. Instead of salicylate 5-hydroxylase, the plasmid harbors three genes annotated as cytochrome P450, hydroxymethylglutaryl-CoA lyase, and formyl-CoA trasferase, probably converting salicylate to gentisate. In this study, we will characterize the three genes and discuss their functions in naphthalene biodegradation of strain NS6^T.

FEMS7-2952
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

PHOTOTROPHIC BIOFILMS IN CUEVA DEL TESORO, RINCON DE LA VICTORIA, MALAGA, SPAIN

V. JURADO[1], H.M. MARIONA[2], H. BERNARDO[1], S.J. CESAREO[1]
[1]INSTITUTO DE RECURSOS NATURALES Y AGROBIOLOGÍA DE SEVILLA- CSIC, Agroquímica- Microbiología Ambiental y Conservación de Suelos, SEVILLA, Spain
[2]UNIVERSIDAD DE BARCELONA, FACULTAD DE FARMACIA, BARCELONA, Spain

Backgrounds

In the last few years Cueva del Tesoro has been investigated regarding the need to control phototrophic biofilms colonizing cave speleothems.

Objectives

Cleaning of caves should be preceded by an inventory of species and testing of treatment protocols due to the fact that biocides have important negative effects in some caves.

The green biofilms in this cave were uncommon and presented some characteristics worthy of study. These communities were triggered by the artificial lighting which is located in selected places along the visitor´s trail. The assemblages were mainly composed of Cyanobacteria (*Friedmannia* sp., *Nostoc* sp., *Phormidium* sp., *Gleocapsa*-like members, etc.) Chlorophyta (*Friedmannia* sp., *Chlorella* sp., *Choricystis* sp.), Rhodophyta (*Cyanidium* sp.), and Bacillariophyta (*Diadesmis contenta*). Some locations develop abundant populations of the bryophyte *Eucladium verticillatum*.

Moreover, three novel bacterial species are being studied: *Acinetobacter thesauricus*, *Bacillus thesauricus* and *Paracoccus speluncae*; and two new fungal species were described: *Aspergillus thesauricus* and *Aspergillus baeticus*

Methods

We tested three cleaning methods: i) Mechanical cleaning with liquid nitrogen, ii) Cleaning with sodium hypochlorite and iii) Cleaning with hydrogen peroxide. The most effective method was hydrogen peroxide which was further used by the restoration company carrying out the works. In addition, a remodelling of the lighting system and installation of new LED lamps in more appropriate places were suggested.

Conclusions

Cueva del Tesoro revealed to harbour a rich biodiversity which suggests that in addition to cleaning and control of the biofilms, some actions are needed to preserve this cave and its microbial diversity.

FEMS7-1178
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TOWARDS DEFINING ESSENTIAL METABOLIC PATHWAYS AND REGULATORY FACTORS INVOLVED IN VIBRIO HARVEYI ADAPTATION IN MARINE ENVIRONMENTS

V. Kaberdin[1,2,3], *I. Montánchez*[1], *A. Hernández Plágaro*[1], *C. Parada*[1], *M. Orruño*[1,3], *I. Arana*[1,3]
[1]*Universidad del País Vasco UPV/EHU, Immunology- Microbiology and Parasitology, Leioa, Spain*
[2]*IKERBASQUE, Basque Foundation for Science, Bilbao, Spain*
[3]*PiE-UPV/EHU, Research Centre for Experimental Marine Biology and Biotechnology, Plenzia, Spain*

Backgrounds

The genus *Vibrio* encompasses a large group of bacteria ubiquitously present in aquatic ecosystems. Previous work has shown that *Vibrio* species possess a high capacity to survive under severe stress conditions including starvation, drastic temperature shifts and solar radiation.

Objectives

Vibrio harveyi was used as a model organism to study the temperature-dependent adaptation of this marine bacterium to starvation under conditions mimicking its natural environments.

Methods

Advanced microscopy along with cell plating techniques and highthroughput gene expression analysis were employed to monitor viability, physiological state as well as morphological and gene expression changes taking place during *V. harveyi* persistence in seawater microcosms.

Conclusions

These experiments revealed that, besides a gradual decrease in size and morphological changes, prolong (> 3-6 days) incubation in seawater at 20-30^{o}C led to a profound decrease in gene expression affecting the central carbon metabolism, major biosynthetic pathways and energy production. The above changes were accompanied by a concomitant increase in expression of genes closely involved in lipid degradation, recycling of amino acids and ancillary mechanisms important for sustaining iron homeostasis and cell resistance to the toxic effect of reactive oxygen species. Furthermore, RNA-seq and microarray data also suggested that some of the adaptation steps apparently involved small regulatory RNAs (e.g. GcvB) known for their essential roles in bacterial responses to stress. Based on the experimental data obtained, we defined the putative mechanisms and major factors likely contributing to the main physiological, phenotypical and gene expression changes that occur during *V. harveyi* adaptation in natural aquatic systems.

FEMS7-1117
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE EFFECTS OF INSULIN AND GLUCOSE ON EXPRESSION OF GENES IN E. COLI MAR OPERON

D. Gumus[1], F. Kalayci[1], E. Yoruk[2], G. Uz[2], A. Bayraktar[2], P. Koseoglu[2], Y. Ofluoglu[2], B. Daylan[2], M. Ang Kucuker[1]
[1]Istanbul Yeni Yuzyil University Medical Faculty, Medical Microbiology, Istanbul, Turkey
[2]Istanbul Yeni Yuzyil University- Faculty of Arts and Sciences, Molecular Biology and Genetics, Istanbul, Turkey

Backgrounds

Microbes and their host environment is always linked dynamically to each other. Host environmental factors are known to modulate virulence factors, antibiotic resistance and growth rate of commensal bacteria.

Objectives

In this study we aimed to investigate the effects of insulin and glucose, as environmental factors, in host on expression of *acrA, acrB, marA, marR, ompF* and *tolC* genes in mar operon in a standard *E. coli*.

Methods

The standard *E. coli* SPC105 strain was cultured in tryptic soya broth (TSB) with or without insulin (20µIU and 200µIU) and/ or glucose (0.1%). Total RNA isolations were performed after 24 hours incubation at 37^0C. Gene expression levels were determined by quantitative PCR. The results were evaluated by relative quantitation method to compare the gene expression levels with 16S rRNA gene expression level. Tukey post hoc-test was used for statistical analysis. Each experiment was replicated at least thrice.

Conclusions

Expression of *acrA* gene was shown to be decreased in the presence of 200µIU insulin. Expression of *marR* gene decreased in 200µIU insulin, 0.1 % glucose+200µIU insulin and 0.1% glucose added TSB. The decrease in *acrA* and *marR were found to be statistically significant.* On the other hand there were no statistically significant difference on expression levels of *acrB, marA, tolC* and *ompF* genes between control medium and insulin or glucose added TSB. In qPCR analysis, melting scores were ranged from 92% to 98%.

It can be suggested that host factors which put together the environment of bacteria in a host may influence the gene expressions associated with antibiotic susceptibilities.

FEMS7-0060
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ARSENIC BIOREMEDIATION FROM WATER BY MULTI-METAL RESISTANT PAENIBACILLLUS JAMILAE ISOLATED FROM URANIUM RICH SUBSURFACE SOIL

D. Kalita[1], S.R. Joshi[1]
[1]North Eastern Hill University, Department Of Biotechnology And Bioinformatics, Shillong, India

Backgrounds

Arsenic contamination is a major health problem of northeast India. The Bengal delta plain consisting of the Ganga–Padma–Meghna–Brahmaputra river basin covering West Bengal, North-east of India and Bangladesh is reported as a highly arsenic-affected alluvial basin in the world with 112 to 657 µg/L in groundwater. Approximately 6.3% drinking water samples had arsenic concentration above the WHO recommended permissible limit of 50µg/L.

Objectives

The current investigation was undertaken to screen the microbial adherence, structure, composition and hydrophilic ligands of exopolysaccharide of Arsenic resistant *Paenibacillus jamilae* and their application to bioremediation.

Methods

A metallophilic bacterium, *Paenibacillus jamilae* isolated from the subsurface soil of Domiasiat and characterized by morphological, biochemical and molecular approaches. Was evaluated for arsenic resistance for its use in bioremediation purposes. The exopolysaccharide of the bacterium was tested for various microbial adherence properties along with the sugar and ligands characterization.

Conclusions

The bacterium showed strong and positive affinity towards the adherence property in the PAT and SAA test. The bacterium was found hydrophilic in nature with the index of 18.25 and 24.01 in adherence test and contact angle measurement respectively. The GC-MS and FTIR chromatogram showed presence of sugar molecules and hydrophilic functional groups, having capability to bind metals insurface.The structure and composition of the exopolysaccharide was inferred by SEM images.In batch culture experiment, *Paenibacillus jamilae* removed87% of arsenic from the synthetic Bangladesh groundwater.

The properties of the bacterium,*Paenibacillus jamilae* can be exploited for bioprospecting for bioremediation of arsenic and other metals from wastewater and groundwater.

FEMS7-2760
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BACTERIAL COMMUNITY STRUCTURE OF THE PACIFIC OCEAN ALONG A TRANSECT FROM ALASKA TO THE ANTARCTIC PENINSULA VIA THE KOREAN PENINSULA

I. Kang[1], *S.J. Yang*[2], *J.C. Cho*[1]
[1]*Inha University, Dept. of Biological Sciences, Incheon, Republic of Korea*
[2]*ChunLab Inc., Seoul, Republic of Korea*

Backgrounds

Bacterial assemblages play crucial roles in biogeochemical cycles in marine environments.

Objectives

In this study, bacterial community structure of the Pacific Ocean was analyzed to better understand the influence of environmental parameters on the composition of marine bacterial assemblages.

Methods

A total of 80 surface seawater samples were collected onboard ARAON, the ice-breaking research vessel. Thirty samples were obtained during a cruise from the Korean Peninsula to Alaska, USA. Another 50 samples were collected during a subsequent cruise from the Korean Peninsula to the Antarctic Peninsula. After removing large-sized particles and organisms, free-living bacteria of water samples were collected onto 0.2-μm pore-sized membrane filters, which were used for DNA extraction. Bacterial community structure was analyzed by pyrosequencing of 16S rRNA gene sequences amplified from extracted DNA samples.

Conclusions

The results showed that bacterial assemblages of surface water of the Pacific Ocean were dominated by *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, and *Cyanobacteria*. The SAR11 and SAR86 clades were prevalent in nearly all samples. The genus *Prochlorococcus* was abundant in oligotrophic areas of low-latitude regions but nearly undetectable in high-latitude regions. The Arctic96BD-19 (ZD0405) clade of the *Gammaproteobacteria*, NS groups of *Bacteroidetes,* and the marine *Roseobacter* clade of the *Alphaproteobacteria* were more abundant in high latitude regions. Ordination analyses based on beta-diversity indices and environmental parameters showed that the community structure of free-living bacteria of the Pacific Ocean changed according to oceanic provinces and was largely influenced by temperature, chlorophyll concentration, and salinity.

FEMS7-3133
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE METRO SYSTEM MAY MODULATE THE SKIN MICROBIOME AND RESISTOME BY ITS ENVIRONMENTAL EXPOSURES AND INNER- AND INTERCITY TRAFFIC FLOWS

K. Kang[1,2], J. Li[1,2], G. Panagiotou[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöl, Systems Biology and Bioinformatics, Jena, Germany
[2]The University of Hong Kong, The School of Biological Sciences, Hong Kong, Hong Kong S.A.R.

Backgrounds

Skin functions as the primary interface between the human body and the external environment, therefore great attention is given to the disinfection of high-density urban environments to limit the spread of pathogens. Yet, we have little understanding of how the built microbiome varies within urban environments and what factors drive the diversity and risk of pathogenicity therein.

Objectives

Hence, we established a metagenomic study regarding human interactions with public transit systems, and built a dynamic and global view of the microbial community and its potential health risks shaped by the traffic.

Methods

We used shotgun metagenomic sequencing to profile the palm microbiome after contact with handrails within the Hong Kong Mass Transit Railway (MTR) system. Our sampling has covered different times (AM vs PM commute), over the course of 3 days and targeted distinct lines (8) which serve different urban centers in the city.

Conclusions

Intraday sampling time was identified as the primary determinant of the variation and recurrence of the community composition, whereas human-associated species and clinically important antibiotic resistance genes (ARGs) were captured as PM signatures. Line-specific signatures were notably correlated with line-specific environmental exposures and civic characters, and losing their discriminatory power over time in the operating hours. For instance, high uniqueness was observed in the most topologically isolated line, which runs by a polluted river. The aquatic species and indicator organisms for sewage could also be identified as signature species of this line, in AM communities in particular. The only cross-boundary line appeared as an outlier in most analyses including the community diversity and dissimilarity. This line had the 2nd highest abundance of clinically important ARGs and community-wide dissemination potential, and the greatest intraday increments in the abundance of ARGs and clinically important ARGs (33.5% and 24.1%), suggesting a potential cross-boundary ARG transmission, especially tetracycline resistance.

FEMS7-1897
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ISOLATION OF UNCULTURED ANAEROBIC BACTERIAL SPECIES FROM FECES OF KOREAN ADULTS

W. Kang[1], N.R. Shin[1], D.W. Hyun[1], P.S. Kim[1], H.S. Kim[1], J.Y. Lee[1], E.J. Tak[1], H. Sung[1], J.W. Bae[1]
[1]KyungHee University,
Department of Life and Nanopharmaceutical Sciences and Department of Biology, Seoul, Republic of Korea

Backgrounds

Although studies on human gut microbial community based on next-generation sequencing techniques have been actively conducted recently, most of bacterial members in the human gut microbiota remain unculturable and have not been given a valid name. Expanding the availability of the human gut bacterial species by isolating and preserving will greatly contribute to improvement of insight into host-microbial interaction and development of therapeutic strategy focusing on the indigenous symbiont.

Objectives

The objective of this study was cultivation and characterization of taxonomic position of bacterial species resident in the human intestinal tract that have never been isolated.

Methods

Fecal samples from six Korean adults were homogenized and diluted in phosphate-buffered saline. The diluted solutions were spread onto Gifu-Anaerobic Medium supplemented with vitamin B solution, Trypticase-Soy Yeast-extract Agar and Brain-Heart Infusion agar media and were incubated for 3 days at 37 °C in an anaerobic chamber.

Conclusions

Five strains (2 strains of *Parabacteroides* sp., a strain of *Bacteroides* sp., *Anaerostipes* sp. and *Blautia* sp.) of novel bacterial species candidates were identified from the pure cultures by 16S rRNA gene sequence.

FEMS7-2894
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

IN VITRO ACTIVITIES OF COLISTIN IN COMBINATION WITH DORIPENEM, DOXYCYCLINE AND RIFAMPIN AGAINST MULTI-DRUG RESISTANT ISOLATES OF PSEUDOMONAS AERUGINOSA AND ACINETOBACTER BAUMANII

E. Karaaslan[1], S. Dosler[2]
[1]Bezmialem University- Faculty of Medicine, Medical Microbiology, Istanbul, Turkey
[2]Istanbul University- Faculty of Pharmacy, Pharmaceutical Mirobiology, Istanbul, Turkey

Backgrounds

Pseudomonas aeruginosa and *Acinetobacter baumannii* are nonfermentative Gram negative bacilli which are two of the leading causes of life threatening nosocomial infections and also public health threat. Besides causing infections with high mortality and morbidity rates, these two organisms are intrinsically resistant to most antibiotics and also excellent at developing new resistance mechanisms. During these times of antibiotics pipeline, developing combination therapies against these bacteria have become crucial.

Objectives

To test the effectiveness of combination therapies against clinically isolated and multi drug resistant strains of *P. aeruginosa* and *A.baumannii*.

Methods

Total 51 clinical isolates (23 *P. aeruginosa* and 28 *A.baumannii*) initially screened for the assessment of their resistance against colistin, doripenem, doxycycline, gentamicin, ciprofloxacin, ceftazidime, rifampin with broth microdilution assay. According to their resistance profiles 20 isolates (10 *P. aeruginosa* and 10 *A.baumannii*) were selected and colistin-doxycyclilne, colistin-doripenem and colistin-rifampin combinations were tested with checkerboard method. Also on select isolates, time kill curve experiments were carried out.

Conclusions

According to MIC values, all isolates were resistant to almost all antibiotics except for colistin. In combination studies, synergistic effects were mostly seen with colistin-doxycycline against *P. aeruginosa* (90%) and *A. baumonii* (60%), followed by the colistin-doripenem combinations (30%). In TKC studies, synergism was observed with almost all expected combinations especially with colistin-doxycycline and colistin-doripenem, even at 1/4xMIC values were used ın combinations.

FEMS7-1100
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EVALUATING COMPOUNDS FOR ABILITY TO REDUCE UREOLYTIC ACTIVITY IN ANIMAL MANURE SLURRY

H. Karring[1], J.J. Sigurdarson Gade[1], S. Svane[1]
[1]University of Southern Denmark,
Department of Chemical Engineering- Biotechnology and Environmental Technology, Odense, Denmark

Backgrounds

Utilizing pig manure slurry as fertilizer in agriculture presents the challenge of controlling the microbiologically driven conversion of urine-derived urea to ammonia (NH_3) and the following volatilization of NH_3. Fertilizer nitrogen lost as NH_3 causes significant environmental and economic problems e.g. decreasing fertilizer value and deposition of excess nitrogen in the environment.

Objectives

The objectives of this study are to develop a medium-throughput bacterial and enzyme assay for screening compounds for anti-ureolytic activity and identify a method for reducing urea hydrolysis in manure slurry.

Methods

The assay relies on the absorbance change of a pH-indicator as a result of the change in pH caused by ureolytic NH_3 production. The absorbance was recorded with a plate reader and used to evaluate the ability of 60 compounds to reduce urea hydrolysis. The chemicals were screened at different concentrations for ability to reduce the ureolytic activity of both *K. pneumonia* and purified jack bean urease. Some of the compounds which displayed anti-ureolytic effect were further tested on pig manure slurry and the ureolytic activity quantified by the Kjeldahl method.

Conclusions

Using 96-well plates for the assay allows for the simultaneous triplicate test of 8 compounds at three different concentrations. A third of the tested compounds were found to have an effect against ureolytic bacteria while app. 20% worked against jack bean urease. A few compounds investigated in this assay showed reduction of ureolytic activity in pig manure slurry.

FEMS7-2458
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EFFECT OF DIFFERENT HONEY TYPES AND THEIR COMBINATIONS WITH ESSENTIAL OILS ON BACTERIAL BIOFILM FORMATION AND QUORUM SENSING

L.A. Zsadon[1], E.B. Kerekes[2], C. Vágvölgyi[2], J. Krisch[1]
[1]University of Szeged- Faculty of Engineering, Institute of Food Engineering, Szeged, Hungary
[2]University of Szeged- Faculty of Science and Informatics, Department of Microbiology, Szeged, Hungary

Backgrounds

Biofilm formation represents a serious problem by lowering the shelf-life of foods thereby affecting food quality and safety. Quorum sensing (QS) is known to be crucial for biofilm formation. By targeting QS, the growth of bacteria is not influenced which would lead to selection pressure and to development of resistance.

Objectives

The aim of the present study was to test the effect of different honey types and honey-essential oil combinations on the QS of two pigmented bacteria.

Methods

Honey from acacia, linden, sunflower and silk grass were tested in different concentrations for their anti-QS and anti-biofilm forming effect on *Chromobacterium violaceum* 85 WT and *Serratia marcescens*. Honey solutions were supplemented with essential oils (EOs) of marjoram and clary sage at different concentrations and their effect was also evaluated. Pigment production of these bacteria is under QS regulation therefore, violacein and prodigiozin were extracted and quantified. Besides pigment production, changes in the culture cell numbers were also determined. Biofilm formation was investigated by crystal violet assay.

Conclusions

Results show that with low concentrations of honey solutions the QS could be inhibited without any change in the colony cell number. Also the inhibitions of QS lead to the disruption of biofilm formation. In general, addition of EOs enhanced the effect of honey solutions from which sunflower honey proved to be the most efficient in both tests. In conclusion, each tested honey proved to be good anti-QS and anti-biofilm forming agent which effect could be synergistically increased with the addition of the tested EOs.

FEMS7-2262
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE ROLE OF MINERAL SCAFFOLDS IN BIOFILM DEVELOPMENT AND ANTIBIOTIC RESISTANCE

A. Keren-Paz[1], V. Brumfeld[2], Y. Oppenheimer-Shaanan[1], I. Kolodkin-Gal[1]
[1]Weizmann Institute of Science, MOlecular Genetics, Rehovot, Israel
[2]Weizmann Institute of Science, Department of Chemical Research Support, Rehovot, Israel

Backgrounds

Bacterial multicellular communities, called biofilms, have tremendous impact on human life and health. In biofilm, the bacteria are protected from environmental insults and assaults, and their attachment to different hosts is improved. Moreover, biofilm can be up to 1,000 times more resistant to antibiotics than planktonic (free-living) bacteria.
The molecular mechanisms supporting biofilm resistance are poorly understood. Organic extracellular matrix production, the hallmark of biofilm formation, has been extensively studied, and its role in cell–cell and cell–substrate adhesion is well established. However, the possible existence of **mineral scaffolds** providing rigidity to bacterial biofilms has been largely neglected.

Objectives

We recently discovered that controlled deposition of a calcium carbonate scaffolds can structurally support morphogenesis of bacterial colonies in both Gram-Negative and Gram-positive bacteria. In this study, we characterize the contribution of those calcite scaffolds
to biofilm development and antibiotic resistance.

Methods

To study this novel aspect of biofilm development, we propose a new method to analyze spatio-temporal accumulation of minerals within biofilms using MicroCT X-Ray.

Conclusions

We propose a central role of biomineralization molecular pathways in biofilm formation, and show that genetically manipulating or chemically inhibiting enzymes catalyzing crucial steps in biomineralization has a dramatic effect on biofilms of different bacterial species. Furthermore, we examine the contribution of enzymes involved in biomineralization to antibiotic resistance of pathogenic and beneficial biofilms, and demonstrate that chemical interference with those proteins can overcome antibiotic resistance within biofilms.

Our overall research sheds light on a novel aspect of biofilm development and suggests novel targets for anti-biofilm drugs.

FEMS7-3131
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIOTA DIVERSITY AND JEJUNUM MORPHOLOGY IS NOT AFFECTED BY HIGH-DOSE VOLATILE OIL COMPOUNDS

J.C. Kesselring[1], V.A. Sattler[2], I. Schantl[3], N. Reisinger[4], K. Teichmann[4]
[1]Biomin Holding GmbH, FD G2P, Getzersdorf, Austria
[2]BIOMIN Research Center, GutOmics, Tulln, Austria
[3]BIOMIN Research Center, FD G2P, Tulln, Austria
[4]BIOMIN Research Center, Cell Biology, Tulln, Austria

Backgrounds

Organic chemical compounds from plants are gaining popularity as growth promoting agents in animal husbandry. Among the suggested modes of action, antimicrobial effects were described.

Objectives

A feed additive containing the volatile oil compounds L-menthol, D-carvone and carvacrol was fed in high dose (up to 10x recommended dose) to weaned piglets and broilers to assess potential effects on the microbiota diversity and gut morphology.

Methods

Jejunum and colon digesta as well as mid jejunum were sampled for microbiota assessment and for the appraisal of gut morphology. Total genomic DNA was extracted with the QIAamp DNA Stool Mini Kit (Qiagen, Germany) after lysozyme pretreatment and bead-beating. The V3 region of the 16S rRNA gene was amplified and analyzed by DGGE. For evaluation of the gut morphology, paraffin embedded tissue (mid-jejunum) was stained with the Periodic Schiff Assay staining method and evaluated under a light microscope.

Conclusions

On average broiler jejunum samples had 26 ± 4.1 dominant bands and piglet colon samples had 35 ± 3.6 dominant bands in their DGGE profiles. Cluster analysis showed that the phytogenic additive did not have any influence on the bacterial diversity of dominant members of the broiler jejunum microbiota or the piglet colon microbiota. There was no significant effect of any treatment on villus length, crypt depth, V:C ratio and total goblet cell number per villus compared to the untreated control.

The phytogenic feed additive did not negatively affect any of the tested parameters, confirming its safety for the target species even in high dose.

FEMS7-0752
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DIVERSITY OF ANTIBIOTIC RESISTANCE GENES IN GRAM-NEGATIVE BACTERIA FROM AQUATIC ENVIRONMENTS IMPACTED BY HOSPITAL AND HOUSEHOLD WASTEWATER

F.A. Khan[1], B. Söderquist[2], J. Jass[1]
[1]The Life Science Center - Biology, School of Science and Technology- Örebro University, Örebro, Sweden
[2]School of Medical Sciences, Faculty of Medicine and Health- Örebro University, Örebro, Sweden

Backgrounds

Enterobacteriaceae and non-fermentative Gram-negative rods such as *Acinetobacter* and *Pseudomonas* species are one of the major causes of nosocomial infections. Most clinically significant Gram-negative bacteria harbor plasmids with antibiotic resistance genes (ARGs) that are mobile due to horizontal gene transfer. There is growing evidence that ARGs are ubiquitous in natural aquatic environments.

Objectives

The main objective was to evaluate the repertoire of ARGs in a Gram-negative bacterial population from a hospital, wastewater treatment plant (WWTP), recipient river, and lake in Sweden, and to identify the possible route of dissemination into the environment.

Methods

Selective coliform agar was used to grow and enrich for Gram-negative bacteria from the water samples. High-throughput quantitative PCR was performed to compare the diversity of ARGs in Gram-negative bacteria from river and lake impacted by household and hospital wastewater.

Conclusions

The genes conferring resistance to aminoglycosides, fluoroquinolones and tetracycline were ubiquitous in all sites tested, while macrolide and multidrug efflux pumps were not found upstream or in the recipient lake. Class A and C β-lactamase genes were present in all the sites, however, metallo-β-lactamase genes (*bla*$_{IMP}$ and *bla*$_{VIM}$) were present in the hospital wastewater but not in the WWTP. Interestingly, *bla*$_{VIM-1}$ was found in the river receiving wastewater from WWTP. Additionally, the Class D β-lactamase genes were found at all sites except upstream in the river suggesting they originate from the city wastewater. Further analysis of ARGs in the community is needed to assess route and mobility of ARGs in the environment.

FEMS7-0422
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

A UNIQUE APPLICATION OF MICROBIAL PEROXIDASES FOR RECALCITRANT PLASTIC WASTE DEGRADATION

N. Khatoon[1,2], *I. Ali*[3]
[1]*Duke university USA, department of environmental engineering, durham, Pakistan*
[2]*Quaid i azam university, department of Microbiology, Islamabad, Pakistan*
[3]*quaid i azam university, microbiology, islamabad, Pakistan*

Backgrounds

Polymeric compounds become challenging due to their persistent nature when released into the environment as a waste. Oxidative enzymes play significant role in biodegradation of recalcitrant materials. Fungi are important among microorganisms for production of extracellular enzymes

Objectives

Bodegradation of the recalcitrant polymer by catalytic oxidation through microbial peroxidase is novel approach for clean aquatic environment

Methods

Maximum enzyme production from indigenous isolates was observed 8.76µl/100ml by using vertyl alcohol as inducer. Statistical analysis (ANOVA) indicate the significance of enzyme production model and components on the basis of F value and P value <0.05 for rate of peroxidase activity. A band of 46 KDa was observed for lignin peroxidase. The Fourier transform infrared (FTIR) spectroscopy of enzyme treated plastic films revealed the structural changes as compared to control (without enzyme treatment. The significant change was observed in peak at wavelength 7866.09 cm^{-1} which attributes to C-H bonding. Application of enzyme on different substrate (polystyrene, polypropylene, polyvinyl chloride) indicates the different level of degradation. The structural changes on the on the surface and crakes and hole clearly showed degradation occur.

Conclusions

Peroxidase enzymes have the potential for biodegradability of recalcitrant plastic waste and can be used for plastic waste treatment at large scale.

FEMS7-0369
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ANALYSIS OF MICROBIOTA ON OCTOPUS VARIABILIS IN SOUTH KOREA

B.S. Kim[1], J.J. Lee[1], T.Y. Kim[1]
[1]Hallym University, Life Science, Chuncheon, Republic of Korea

Backgrounds

Octopus variabilis is one of popular seafood as a healthy food in South Korea. However, it can cause food poisoning when ingested as raw material.

Objectives

Analysis of microbiota on *O. variabilis* can improve the understanding of food poisoning risk and the management of product.

Methods

A total of 38 *Octopus variabilis* samples were collected from four sites in November and August. The microbiota on *O. variabilis* was analyzed by the Illumina Miseq based on 16S rRNA gene, and the quantification of bacteria was performed by real-time PCR. In addition, we analyzed the difference of microbiome between artificial infected model by *Vibrio vulnificus* and non-infected model.

Conclusions

The diversity of microbiota was higher in summer samples than that in winter samples. Bacteroidetes was predominant phylum in summer samples, whereas Proteobacteria was predominant in winter samples. *Coenonia* and *Mycoplasma* were dominant genera in summer samples, whereas *Burkholderia* and *Psychrobacter* were dominant genera in winter. The difference of microbiota among sampling regions was also detected. In infected experiment, the proportion of *V. vulnificus* was increased only at room temperature. However, the proportion of *V. vulnificus* was similar along storage time at 4℃, and the functional genes of microbiome were shifted similar between infected and non-infected model along time. These results indicate that the indigenous microbiota on *O. variabilis* can protect the colonization of *V. vulnificus* during storage at 4℃. Results in this study will help to understand the food poisoning caused by octopus ingestion and the management of product.

FEMS7-1624
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

GENOME SEQUENCING AND COMPARATIVE GENOMICS OF THE ORGANOPHOSPHORUS PESTICIDES-DEGRADING BACTERIUM, SPHINGOBIUM SP. EP60837

B.Y. Kim[1], *C.M. Lee*[2], *S. Yang*[1]
[1]*ChunLab- Inc., Bioinformatic institute, Seoul, Republic of Korea*
[2]*National Institute of Agricultural Sciences- RDA, Metabolic Engineering Division, Jeonju, Republic of Korea*

Backgrounds

Organophosphorus pesticides, which are highly poisonous and cause serious concerns over food safety and environmental pollution, are still widely used for pest insect control. Environment-friendly bioremediation is increasingly important in agriculture.

Objectives

The bacterium, designated EP60837, which showed the high activity of degradation of organophosphorus pesticides, including ethoprophos, cadusafos and chlorpyrifos, was isolated from agricultural soil in Korea,The bacterium, designated EP60837, which showed the high activity of degradation of organophosphorus pesticides, including ethoprophos, cadusafos and chlorpyrifos, was isolated from agricultural soil in Korea,

Methods

Here, we performed complete genome sequencing using PacBio RSII system and bioinformatic analysis. Also comparative genome analysis of *Sphingobium* sp. EP60837 with closely related strains was done.

Conclusions

The genome of strain EP60837 consists of two chromosome and two plasmids. An array of genes related to the degradation of pesticides was identified by whole genome analysis. The genome sequence of the strain EP60837 provides basic information for wider exploitation of bioremediation of organophophorus pesticides contaminated soil with soil microorganism

Acknowledgement: This work was supported by grants from National Institute of Agricultural Sciences (PJ-01045703), Rural Development Administration, and The Strategic Initiative for Microbiomes in Agriculture and Food (No. 914008-04) of Agricultural Microbiome R&D Program, Republic of Korea.

FEMS7-1522
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TRANSCRIPTOME ANALYSIS OF HALOARCHAEOBIUS SP. HME9146 IN RESPONSE TO SALT AND TEMPERATURE STRESS

H. Kim[1], H. Kang[1], I. Cha[1], K. Joh[1]
[1]Hankuk University of Foreign Studies, Department of Bioscience and Biotechnology, Yongin, Republic of Korea

Backgrounds

Solar salterns are artificial ponds for the production of salt from seawater and can be found halophilic archaea. The haloarchaea are dominant and survive from high salinity and temperature by intense sunlight in solar salterns.

Objectives

In this research, we performed RNA sequencing (RNA-seq) to investigate the transcriptome of a halophilic archaeal strain *Haloarchaeobius* sp. HME9146 in response to salt and temperature stress.

Methods

The strain *Haloarchaeobius* sp. HME9146 was isolated from a solar saltern in Republic of Korea. The RNA samples were extracted from strain HME9146 grown under different salt (2.5 M NaCl or 4.0 M NaCl) and temperature (30 ℃ or 45 ℃) conditions. The four samples were designated A (30 ℃ and 2.5 M NaCl), B (30 ℃ and 4.0 M NaCl), C (45 ℃ and 2.5 M NaCl) and D (45 ℃ and 4.0 M NaCl), and sequenced using the Illumina HiSeq 2500 platform.

Conclusions

The comparison of A and B samples by RNA-seq reveal that 962 genes were up-regulated and 897 genes were down-regulated by salt stress at 30 ℃. The comparison of C and D samples reveal that 3 genes were up re-regulated and 18 genes were down-regulated by salt stress at 45 ℃ (>2 folds). The comparison of four samples revealed that genes involved in lipid homeostasis and acetoin catabolism were down-regulated under high salt concentration in both temperature conditions (30 ℃ and 45 ℃). In addition, a considerable proportion of the significantly differently expressed genes identified in this study are novel. These data provide a crucial resource that may determine specific response to salt and temperature stress in *Haloarchaeobius* sp. HME9146.

FEMS7-1519
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COLLAPSE OF HUMAN SCALP MICROBIOME NETWORK IN DANDRUFF AND SEBORRHEIC DERMATITIS

H.J. Kim[1], T. Park[2], W.J. Sul[1]
[1]Chung-Ang University, Department of Systems Biotechnology, Anseong, Republic of Korea
[2]Amorepacific R&D center, Safety Research, Youngin, Republic of Korea

Backgrounds

Human scalp accommodates diverse bacteria and fungi that influence health condition. Some scalp commensal microorganism are widely known cause of most scalp diseases. However, there is still controversy regarding the microbial species responsible for dandruff and seborrheic dermatitis.

Objectives

We questioned i) whether there was any difference between diseased scalps and normal scalps with respect to their microbial community structure and diversity, and ii) whether the bacterial and fungal network collapsed in diseased scalps compared to normal scalps. In this work, we investigated the difference in the scalp microbiome of three different groups, namely normal (n=45), dandruff (n=28), and seborrheic dermatitis (n=29), in Korea.

Methods

Scalp microbiota were sampled, and the bacterial 16S rRNA gene and fungal ITS1 region were characterized using Illumina MiSeq platform.

Conclusions

PCoA revealed appreciably distinct bacterial and fungal communities among normal and disease groups. The disease status was the major factor used to separate bacterial (ANOSIM; R = 0.382; P = 0.001) and fungal communities (R = 0.548, P = 0.001) from different groups. We used SPIEC-EASI to construct a correlation network among the microbiome resident on the scalp. As a result, the disease groups showed a smaller and less complex network of bacterial and fungal communities than the normal group, suggesting that the healthy scalp microbiota stability had collapsed. Our results will not only provide insight on finding the key-stone species associated with scalp diseases but will also suggest shift in microbial communities.

FEMS7-1537
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SCREENING AND ISOLATION OF HIGH EFFICIENCY MARINE BACTERIA TO DEGRADE CRUDE OIL

H.S. Kim[1], J. Kim[2], S.S. Lee[1]
[1]Kyonggi University, Biological engineering, Suwon, Republic of Korea
[2]Kyonggi University, Life Science, Suwon, Republic of Korea

Backgrounds

The crude oil spill accident sometimes occurred in the ocean. And then, spilled crude oil made serious occurred pollution in ocean and coastal area. Crude oil spill was very common in the marine habitats which effect on the marine. The pollution was mainly remediated by natural remediation such as evaporation, spreading, dispersion.

Objectives

To date, oil-degrading bacteria were focused in biological remediation of crude oil pollution.

Methods

This study is to screen high efficient bacteria among 125 marine bacteria strains. Through crude oil degrading test for 2 weeks with Bushnell Hass (BH) media in 28°C, pH 7.0 and aerobic condition (150 rpm shaking incubator), we identified high efficient 5 strains such as *Gordonia* sp. Co 17, *Albirhodobacter*, *Rhodococcus*, and *Microbacterium*.

Conclusions

To determine the optimal condition for crude-oil degradation, highest efficiency bacteria *Gordonia* sp. Co 17 degraded average 84.0 % of n-alkanes (C_8 to C_{32}) for 7 days and PAHs and cyclo- compounds during 14 days through GC-FID and GC-MS analysis. From microscopic observation, the strain *Gordonia* sp. Co 17 was found in the oil phase in the degradation test, but they were not emulsified. *Gordonia* sp. Co 17 is tolerance up to for 8 % (w/v). The *alk*B gene known as alkane hydroxylase is important enzyme for alkane degradation pathway, it was confirmed in *Gordonia* sp. Co 17 by PCR. Based on the study, the *Gordonia* sp. Co 17 could be a potential strain for the effective remediation on the crude oil pollution in the ocean and coastal area.

FEMS7-1531
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SEDIMENTITALEA TODARODIS SP. NOV., ISOLATED FROM THE GUT OF A JAPANESE FLYING SQUID

H. Kim[1], D.W. Hyun[1], J.Y. Lee[1], P.S. Kim[1], T.W. Whon[1], W. Kang[1], N.R. Shin[1], E.J. Tak[1], H. Sung[1], J.W. Bae[1]
[1]Kyung Hee University,
Department of Life and Nanopharmaceutical Sciences and Department of Biology, Seoul, Republic of Korea

Backgrounds

The studies of bacterial luminescence systems and quorum sensing using *Vibrio harveyi* and *V. fischeri* have considerably expanded our knowledge of microbial interactions in squid. However, symbiotic interactions between gut microbes and animals in cephalopods have received little attention.

Objectives

This study focused on identifying novel microorganisms in the gut of Japanese flying squid which may affect host physiology, either beneficially or detrimentally, and engaged in interactions with other gut microbes to modify the physiologic effects of marine environments on squid.

Methods

The gut of a Japanese flying squid, *Todarodes pacificus*, collected from the East Sea of Korea, was used as a source of bacterial isolation. We performed phylogenetic, physiological, biochemical, chemotaxonomic and genotypic characteristics of the isolate and its reference strains.

Conclusions

A novel Gram-negative, motile, aerobic and rod-shaped *Alphaproteobacterium*, designated strain KHS03^T, shared 97.4 % sequence similarity of the 16S rRNA gene with type strain of genus *Sedimentitalea*. Strain KHS03^T grew optimally at 25 °C and pH 7 in the presence of 1–2 % (w/v) of NaCl. The major cellular fatty acid was $C_{18:1}$ *ω7c* (82.5 %). The primary isoprenoid quinone was ubiquinone-10. Polar lipids comprised diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, an unidentified aminolipid and two unidentified lipids. The genomic DNA has 59.9 mol% G+C content. DDH showed that the isolate shares 17.1 % ± 2.3 % (17.0 % ± 1.9 %) genomic relatedness with *S. nanhaiensis* NH52F^T. Here, we suggest that strain KHS03^T is a novel species of the genus *Sedimentitalea*, for which the name *Sedimentitalea todarodis* is proposed.

FEMS7-2189
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COMPARATIVE ANALYSIS OF SKIN MICROBIAL COMMUNITY BETWEEN YOUNG AND OLD WOMEN

J.J. Kim[1], H. Kim[2], T. Park[2], S. An[2], H.J. Kim[1], W.J. Sul[1]
[1]Chung-Ang University, Department of Systems Biotechnology, Anseong-si, Republic of Korea
[2]Amorepacific R&D center, Safety Research team, Yongin, Republic of Korea

Backgrounds

Skin is the largest organ in human body with diverse microbial community and influenced by multiple factors such as sex, age, skin surface pH, sebum content, and stratum corneum hydration, especially age is an important factor because other factors vary with it. However, relationship between age and skin microbiome is not yet understood.

Objectives

The purpose of this study is to investigate differences in the skin bacterial community and examine predictive functional profiling for the young and old group.

Methods

A total of 73 samples were recruited from young group (n=48) and old group (n=25) of Chinese women, and 16S rRNA gene sequencing was performed using Illumina MiSeq platform. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to predict metagenome functional contents.

Conclusions

PCoA shows that the bacterial communities of the young group and old group were appreciably distinct. *Chryseobacterium*, *Propionibacterium* and *Enhydrobacter* were dominant in both young and old groups. *Sphingomonas* and *Bacteroides* were more abundant in young group while *staphylococcus* was relatively more in old group. The phylogenetic diversity of skin bacterial communities was higher in the young group than the old group while variation in each group was higher in the old group. Bacterial communities of the two groups were also distinguished in predictive functional profiling and metabolism. From these results, we suggest that differences in bacterial communities and predictive functional profiling across ages will be the cornerstone of therapeutic and cosmetic application associated with human skin aging.

FEMS7-2109
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ECO-FRIENDLY PILOT PLANT-SCALE BIOLOGICAL TREATMENT OF MARINE WASTEWATER FROM LAND-BASED FISH FARM BY HIGH EFFICIENCY MARINE BACTERIA AND MARINE SEDIMENT

J. Kim[1], S. Jeong[1], S. Kim[2], S.S. Lee[1]
[1], Life Science, Suwon, Republic of Korea
[2], Environmental energy engineering, Suwon, Republic of Korea

Backgrounds

Saline wastewater in high concentration is difficult to be biologically treat. It is helpful to reduce cause of the red-tide in the long-term. However, it is required for eco-friendly and effective ways.

Objectives

This study is to apply the pilot plant-scale effective biological treatment of high salinity effluents from land-based fish farm.

Methods

We applied high efficiency NH_3-N removal strain, *Bacillus* sp. KGN1, and PO_4^{3-}-P removal marine strain, *Vibrio* sp. KGP1 to pilot plant-scale biological treatment system with eco-friendly marine sediment. The pilot plant-scale biological treatment consists of an influent tank in 2.5 m^3, an SBR reactor in 4.5 m^3 and an effluent tank in 2.5 m^3 with auto-control system. Using the pilot plant-scale SBR system with marine bacteria and sediment for treatment of marine wastewater from the land-based fish farm improved the treatment performance as indicated by averagely 69.8% R.E. of COD_{Cr} (Influence: 129.1 mg/L, effluence: 39.0 mg/L), 92.7% R.E. of NH_3-N (influence: 5.4 mg/L, effluence: 0.4 mg/L), 73.1% R.E. of T-N (influence: 7.8 mg/L, effluence: 2.1 mg/L), 62.5% R.E. of T-P (influence: 1.6 mg/L, effluence: 0.6 mg/L) at the optimal operation conditions (4h/cycle; 10 min influent period, 2 h 50 min aeration period, 0.5 h settlement, 0.5 h idle& effluent; HRT, 6h; solids retention time (SRT), 12 d). Bacterial community in the eco-friendly marine sludge of the SBR tank was traced change of diversity.

Conclusions

Our system proposed efficient and eco-friendly biological treatment of saline wastewater.

FEMS7-0438
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

RISK FACTOR OF RENAL IMPAIRMENT IN PATIENTS WITH GENITOURINARY TUBERCULOSIS

E.J. Kim[1,2], J.J. Kim[1], J. Kim[1], H. Seong[1], S.J. Lee[1], W.Y. Jeong[1], Y.C. Kim[1,2], I.Y. Jung[1,2], D.H. Oh[1,2], S.J. Jeong[1,2], N.S. Ku[1,2], J.Y. Choi[1,2], Y.G. Song[1,2], J.M. Kim[1,2]
[1]Yonsei University College of Medicine, Department of Internal Medicine, Seoul, Republic of Korea
[2]AIDS research Institute, Yonsei University College of Medicine, Seoul, Republic of Korea

Backgrounds

Genitourinary tuberculosis (GUTB) is a common form of extrapulmonary tuberculosis. Because it leads renal calcification and ureter stricture during healing process, it may have deleterious effects on renal function. Therefore, we investigated to risk factors of renal impairment in GUTB patients.

Objectives

In total, 56 patients with GUTB (51.8% male, mean age 53.2 ± 17.2 years) were enrolled in the study. Renal impairment was developed in 14 (25.0%) patients and end stage renal disease in 4 (7.1%). In univariate analysis, old age (p = 0.011), microscopic hematuria (p = 0.005), proteinuria (p = 0.008), chronic kidney disease (p = 0.003), cardiovascular disease (p = 0.044) and diabetes (p = 0.012) were significantly associated with decreased renal function. In multivariate analysis, microscopic hematuria (odds ratio (OR), 14.334; 95% confidential interval (CI), 1.116-176.152, p =0.038) and old age (OR, 31.748; 95% CI, 2.090-482.189, p =0.013) were independent risk factors of renal impairment in GUTB patients.

Methods

A retrospective cohort study was conducted at a tertiary hospital in South Korea. Patients (>18 years) with GUTB were collected between January 2005 and December 2015. Analysis was performed to identify risk factors associated with renal impairment developed after treatment. Renal impairment was defined as glomerular filtration rate less than 60 ml/min/1.73 m^2.

Conclusions

Microscopic hematuria and old age were independent risk factors of renal impairment in GUTB patients. Therefore, in GUTB elderly patients with microscopic hematuria, regular follow-up of renal function should be needed during and even after the end of treatment.

FEMS7-3163
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL COMMUNITY STRUCTURE AND ITS ECOLOGICAL FUNCTION IN PERRENIALLY ICE-COVERED LAKES OF THE MCMURDO DRY VALLEYS, ANTARCTICA

O.S. Kim[1], M. Kwon[1], J. Lee[1], M. Kim[2], S.G. Hong[2]
[1]Korea Polar Research Institute, Unit of Antarctic K-route Expedition, Incheon, Republic of Korea
[2]Korea Polar Research Institute, Division of Polar Life Sciences, Incheon, Republic of Korea

Backgrounds

Perennially ice-covered lakes in the McMurdo Dry Valleys are chemically stratified with depth and have distinct biological gradients. Despite the long-term studies on these unique environments there remains a paucity of data on microbial community structure and its ecological function in the water columns of these lakes.

Objectives

We investigated bacterial diversity in five different ice-covered Antarctic lakes targeting 16S rRNA genes and ecological function at different depths in Lake Fryxell by metagenomics.

Methods

16S rRNA gene-based pyrosequencing for microbial community structures and shotgun metagenomics by Illumina technology were carried out.

Conclusions

Distinct communities were present in each lake, paralleling the unique biogeochemical characteristics of each environment. Certain bacterial lineages were confined exclusively to a specific depth of each lake. Additionally, we present the metagenomic results to implicate the microbial ecological function in Lake Fryxell.

FEMS7-1517
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SURROUNDING ENVIRONMENT SHAPES GUT MICROBIOME OF FISH AND POSSESS DISTINCT COMPOSITIONAL CHARACTERISTICS FROM OTHER VERTEBRATES

P.S. Kim[1], J.B. Lee[2], M.S. Kim[1], T.W. Whon[1], D.W. Hyun[1], J.H. Yun[1], N.R. Shin[1], M.J. Jung[1], J.W. Bae[1]
[1]Kyung Hee University,
Department of Life and Nanopharmaceutical Sciences and Department of Biology, Seoul, Republic of Korea
[2]National Fisheries Research & Development Institute, Dokdo Fisheries Research Center, Pohang, Republic of Korea

Backgrounds

Gut microbiota contribute to host organism by beneficial effects such as metabolism, immune response as well as ethological aspect. On the one hand, host organisms also select their commensal microbiota that provides advantages to survival or fitness of host. In recent studies, gut microbiota is determined by various factors including diets, habitat, and host phylogeny. So far, the discoveries that suggested with host-microbe interactions and co-evolutionary histories of vertebrate, yet unveiled in the gut microbiota of most of vertebrate species are uncharacterized. Species diversity on fish is greater than any other group of vertebrates and nearly half the total number of vertebrate species, therefore gut microbiota study of fish species attempt us to the dilatational views to understand the entire vertebrate gut microbiota.

Objectives

In this study, we investigated bacterial communities of distal gut and luminal contents from different habitats among more than 230 fishes that can be representatives of 70 species.

Methods

All samples were collected across streams, lakes and sea. Metagenomic DNAs from gut and luminal fluid of each fish species were extracted and then the V1-V3 region of 16S rRNA gene amplified and sequenced by high-throughput NGS technology.

Conclusions

Network-based analysis and beta diversity plots based on Unifrac distances show surrounding environmental features influences bacterial communities of fish. Additionally, fish gut microbiota clustered differently with other vertebrate gut microbiota. Thus, this study suggests deep knowledge to understand unrevealed host-gut microbe interaction in fish and surrounding environments, which can provides circumstantial clue for trends of co-evolution in vertebrate and commensal microbes.

FEMS7-0544
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

GENOMIC RECONSTRUCTION AND METATRANSCRIPTOMIC ANALYSIS REVEAL CARBON-CYCLING BY BACTERIA ASSOCIATED WITH PHYTOPLANKTON BLOOMS IN AN ANTARCTIC POLYNYA

S.J. Kim[1], J.G. Kim[2], S.J. Park[3], J.H. Gwak[2], M.Y. Jung[2], S.K. Rhee[2]
[1]Korea Institute of Geoscience and Mineral Resources, Geologic Environment Research Division, Republic of Korea
[2]Chungbuk National University, Department of microbiology, Cheongju, Republic of Korea
[3]Jeju National University, Department of Biology, Jeju, Republic of Korea

Backgrounds

Polynyas in the Antarctic Ocean are regions of intense primary production, largely by *Phaeocystis antarctica.*

Objectives

Comparative analysis of data from different phases of bloom (peak and decline) and from sea ice-covered ocean provides fundamental insights into the ecology of key heterotrophs associated with phytoplankton blooms in these Antarctic polynyas.

Methods

We sampled Antarctic waters during three cruises (2011 to 2014) and analyzed DNA and mRNA to identify key heterotrophic bacteria active during peak and decline phases of polynya blooms.

Conclusions

Genome reconstruction and comparative metatranscriptomic analyses revealed that *Polaribacter* and Ant4D3 clades were dominant in peak phase and their transcriptional activities were high. Contrastingly, genome binning showed that *Bacteroidetes* and *Gammaproteobacteria* were highly abundant during decline phase. High transcriptional activity for genes in *Polaribacter* encoding metabolism of *P. antarctica's* storage glucan, chrysolaminarin, was found. Transcript abundances in *Gammaproteobacteria* (Ant4D3, SUP05 and SAR92) also indicated widespread utilization of compatible solutes produced by phototrophs. Populations within the SAR92 clade showed transcriptional activity for utilization of both polysaccharides and low molecular weight organic matter; this may account for this clade's abundance both in peak and decline phases. Versatility of *Gammaproteobacteria* associated with large genome size may contribute to their dominance in eutrophic polynya waters, while the SAR11 clade was abundant only in a control site (sea ice-covered oligotrophic ocean). Our results provide insight into niches of key heterotrophic populations engaged in C-cycling and succession of bacterioplankton in Antarctic waters.

FEMS7-2712
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

VAGINAL MICROBIOTA UNDER THE INFLUENCE OF HOST GENETICS AND ITS HEALTH EFFECTS

G. Ko[1], J. Si[1], H. Yu[1], J. Sung[1]
[1]Seoul National University, School of Public Health, Seoul, Republic of Korea

Backgrounds

Recent studies on the various subtypes of vaginal *Lactobacillus* among different ethnic groups suggested host effects on the vaginal microbiota. While the vaginal ecosystem is maintained through mutualistic relationships between the host and the vaginal bacteria, the effect of host genetics on the vaginal microbiota has not been well characterized.

Objectives

The objective of this study is to investigate the effect of host genetics on vaginal microbiota and their association with both vaginal and non-vaginal health conditions such as obesity.

Methods

Here, we examined the heritability of vaginal microbiota and their association with obesity in 542 Korean females, including 222 monozygotic and 56 dizygotic twins.

Conclusions

As expected, the vaginal microbiota significantly varied depending on host menopausal status and bacterial vaginosis, with which the abundance of both *Lactobacillus* and *Prevotella* was strongly associated. Host body mass index (BMI) was also significantly associated with the diversity of vaginal microbiota. Subsequent candidate gene analysis showed that genetic variants of interleukin-5 were strongly associated with the abundance of *Prevotella* sp. Finally, high-fat diet increased the diversity of vaginal bacteria including *Prevotella* in mice, and the transfer of vaginal microbiota promoted metabolic endotoxemia.

FEMS7-2339
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DISTINCT SUBSTRATE CONSUMPTION PATTERNS IN THE MARINE BACTERIUM ALTEROMONAS MACLEODII DURING DEGRADATION OF MACROALGAL POLYSACCHARIDES

H. Koch[1], T. Schweder[2], A. Dürwald[2], M. Simon[1], M. Wietz[1]
[1]University Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany
[2]E.M.A. University of Greifswald, Department of Pharmaceutical Biotechnology, Greifswald, Germany

Backgrounds

Marine phototrophs perform 50% of the global net primary production, forming the basis of the marine carbon cycle. In phototrophic macroalgae, a major fraction of the fixed CO_2 is converted to polysaccharides that serve as storage compounds but also represent important substrates for marine heterotrophic bacteria.

Objectives

To get deeper insights into the bacterial degradation of macroalgal polysaccharides, we studied the globally distributed *Alteromonas macleodii*, a specialist in polysaccharide utilization by encoding a variety of carbohydrate-active enzymes (CAZyme). In this study, we perform physiological and transcriptomic analyses of *A.macleodii* strain 83-1 grown on the macroalgal polysaccharides laminarin, pectin and alginate as sole energy and carbon sources.

Methods

To analyze the effect of mixed substrates on the metabolism of *A.macleodii* 83-1, we incubated the culture in medium containing a mixture of these polysaccharides mimicking natural systems where several polymers occur in concert.

Conclusions

The incubation experiments revealed two distinct growth phases relating to substrate preferences. In the first growth phase laminarin was completely consumed and cells grew to maximum density. In the second phase alginate and pectin were used simultaneously, however without detectable net growth. To identify the molecular mechanisms causing these distinct phases transcriptomic and proteomic analyses are underway. Using comparative genomics, we furthermore show that *A.macleodii* 83-1 and closely related strains have a distinct CAZyme repertoire comparable to other polymer degrading specialists like Flavobacteria.Overall, our results indicate that *A.macleodii* exhibits remarkable genetic and physiological adaptations to complex polymer degradation, a process of great importance in the marine carbon cycle.

FEMS7-2321
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EFFECTIVENESS OF DIFFERENT DISINFECTANTS ON BIOFILM LAYER GENERATED BY MULTI-DRUG RESISTANT MICROORGANISMS

E. Kocoglu[1], B. Guler[2]
[1]Istanbul Medeniyet University School of Medicine, Department of Microbiology, Istanbul, Turkey
[2]Abant Izzet Baysal University- Institute of Health Sciences, Department of Microbiology, Bolu, Turkey

Backgrounds

The major problem with infections caused by multidrug-resistant microorganisms is that treatment options are inadequate. Biofilms cause an increase in morbidity and mortality in patients having nosocomial infections.

Objectives

The aim of this study was to compare the effectiveness of five different disinfectants in different concentrations on biofilm layer formed by multidrug resistant microorganisms.

Methods

For the study, strains isolated from patients having nosocomial infection were used. Enterococcus faecalis ATCC 29212 strain was used as positive control, and Staphylococus aureus ATCC 25923 strain known not to form biofilm as negative control. Sterile stainless steel rods measuring 0.1 mm x 10 mm were used as a model for biofilm formation, and sterilized polystyrene microplates were used as a plastic surface model. Biofilm formation on the model was provided. The biofilm layers were exposed to concentrations of orthophthaldehyde (OPA) 0.55%, sodium hypochlorite 500 and 5000 ppm, iodine 10%, ethyl alcohol 60 and 70%, and citric acid 10 and 30% for 3, 5 and 15 min. The experiments were repeated twice for each contact time, temperature and test condition. A 5 log reduction in the number of microorganisms was accepted to be effective for disinfectant.

Conclusions

OPA, sodium hypochlorite, and ethyl alcohol were found to be effective on the biofilm layer. While the concentration of 10% iodine was found to be ineffective in 3 and 5 min, it was found to be effective when exposed for 15 minutes. Citric acid were found to be ineffective both on the strains forming biofilm layer, on the positive control strains and on the strains that did not form biofilms. According to our findings citric acid in the concentrations used in the experiment has no disinfectant activity in the presence of biofilm, and Iodine requires at least 15 minutes of contact time for efficacy on biofilm layer.

FEMS7-2043
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CHANGES IN FATTY ACIDS COMPOSITION IN SACCHAROMYCES CEREVISIAE CELLS PROTECTED BY RESVERATROL UNDER ETHANOL STRESS

I. Kolouchová[1], O. Maťátková[1], J. Masák[1], T. Řezanka[2]
[1]University of chemistry and technology Prague, Department of Biotechnology, Prague, Czech Republic
[2]Czech Academy of Sciences, Institute of Microbiology, Prague, Czech Republic

Backgrounds

Saccharomyces cerevisiae is one of the most useful yeasts used in biotechnology. It is utilized in ethanol production as well as in winemaking. During the fermentation process, S. cerevisiae is subjected to high ethanol stress, which influences the metabolisms of the cells as well as the biomass growth.

Objectives

The research is focused on *S. cerevisiae* protection by known antioxidant resveratrol as a modulator of ethanol stress. Two traditional biotechnological yeast species of *S. cerevisiae* were exposed to different ethanol and resveratrol concentrations.

Methods

The determination of fatty acid profile (by GC-MS with SP-2380 column), lipid peroxidation products (as TBARS) and changes in the activity of superoxide dismutase were used to determine the degree of ethanol induced cell damage and the resveratrol protective properties.

Conclusions

The results showed that resveratrol has not only the ability to mitigate the impact of ethanol on yeasts, but also to improve their ethanol tolerance.

FEMS7-2303
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

PHYLOGENY AND ENVIRONMENTAL FACTORS DETERMINE METABOLIC ACTIVITIES OF SOIL ACTINOBACTERIA

J. Kopecky[1], Z. Kamenik[2], J. Novotna[1], M. Sagova-Mareckova[1]
[1]Crop Research Institute, Epidemiology and Ecology of Microorganisms, Prague 6, Czech Republic
[2]Institute of Microbiology of the AS CR- v.v.i., Laboratory of Fungal Genetics and Metabolism, Prague 4, Czech Republic

Backgrounds

A complex and structured soil environment provides a variety of resources supporting adaptation, and the microenvironments, where sharing the local gene pool may lead to evolution of site-specific traits.

Objectives

The study was conducted to explore locally specific relationships determined by soil chemistry and vegetation to phylogeny, metabolite profiles and antibiotic activity of cultivable actinobacteria at the sites of their isolation.

Methods

A set of 336 actinobacteria were isolated at ten soil sites, phylogenetically characterized by sequencing the genes for 16S rRNA and the beta-subunit of DNA-dependent RNA polymerase (*rpoB*). The strains were cultivated in submerged culture and the spent media were used to determine the inhibitory activities against *Kocuria rhizophilla*, *Escherichia coli*, and *Saccharomyces cerevisiae*, directly or after solid-phase extraction. In the solid-phase extracted fractions, the profiles of low-molecular weight metabolites were determined by reversed-phase HPLC with UV-VIS diode array detection.

Conclusions

Strains of actinobacteria isolated from the individual sites differed in their phylogeny. The differences were related to soil organic matter contents, but not to soil pH. Activities against the testing strains were related to the *rpoB*-based phylogeny. The profiles of low-molecular-weight compounds differed reflecting both soil organic matter contents and pH at the isolation sites. The relationships of the strains antibiotic activities and profiles of low-molecular weight metabolites to their phylogeny suggested phylogenetic differentiation as the main mechanism determining the strain properties.

FEMS7-1650
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INTERPLAY OF PATIENT MICROBIOME AND HOSPITAL ENVIRONMENT AT INTENSIVE CARE UNIT

K. Koskinen[1,2], M. Mora[1], M.R. Pausan[1], S. Duller[1], C. Högenauer[1], R. Krause[1], C. Moissl-Eichinger[1,2]
[1]Medical University of Graz, Department of Internal Medicine, Graz, Austria
[2]BioTechMed Graz, -, Graz, Austria

Backgrounds

Hospital-acquired infections (HAIs) are a serious problem worldwide. Patient groups most often affected are premature babies, elderly, and patients suffering from immunodeficiency, at intensive care units in particular. The risk is not only related to invasive procedures or inadequate hygiene, but infection can also be transferred directly from patient to patient, via surfaces, equipment and personnel.

Objectives

We conducted a pilot study of one ICU patient (suffering from pneumonia, mechanically ventilated) to recognize the microbial communities, transfer, and significance in the patient's well-being. For consecutive seven days, we sampled the patient skin, trachea, stomach and stool, and environment near the patient.

Methods

16S rRNA gene-based microbial community composition was correlated with clinical parameters, such as fraction of inspired oxygen and arterial oxygen partial pressure.

Conclusions

(Opportunistic) pathogenic bacteria were discovered in patient and environment. However, community structures were distinct in different sampling locations: the patient skin was dominated by *Staphylococcus*, *Anaerococcus* and *Corynebacterium*, stomach aspirate by *Citrobacter*, stool by *Bacteroides*, *Lachnoclostridium*, and *Roseburia,* and tracheal secretion by *Neisseria*, *Gemella*, and *Mycoplasma*. Interestingly, tracheal microbiome shifted on days of poorer oxygenation. Additionally, more diverse tracheal microbiome significantly associated with higher partial pressure of oxygen in arterial blood, suggesting that tracheal microbiome was connected to level of oxygenation. Human skin associated bacteria and (opportunistic) pathogenic bacteria *Acinetobacter*, *Pseudomonas* and *Burkholderia* were detected also in environment. The community structure of environmental samples altered daily reflecting the effect of disinfection, but patient microbiome stayed more constant. During the whole study, environment carried more diversity than patient samples.

FEMS7-3126
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CHARACTERIZATION OF THE ANAEROBIC DEGRADATION OF PHENANTHRENE BY A NOVEL SULFATE-REDUCING BACTERIAL ENRICHMENT CULTURE

I. Kraiselburd[1], T. Bruls[2], R.U. Meckenstock[1]
[1]University of Duisburg-Essen, Biofilm Centre, Essen, Germany
[2]Genoscope Institute of Genomics of the Atomic Energy and Alternative Energies Commission, CEA, Paris, France

Backgrounds

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed contaminants that produce hazardous effects on human health. In PAH-contaminated sites, oxygen is rapidly depleted. Thus, microorganisms able to use these compounds as a carbon source in the absence of molecular oxygen are crucial for their consumption.

Objectives

The aim of this work is elucidating the mechanisms for the anaerobic degradation of phenanthrene by a novel sulfate-reducing enrichment culture (TRIP) obtained from the Pitch Lake in Trinidad-Tobago, the world largest natural asphalt lake.

Methods

The metagenome of the TRIP culture was sequenced and annotated at the Microbial Genome Annotation & Analysis Platform, Genoscope. The analysis of the metagenome sequences included a phylogenetic binning of bacterial genomes followed by a metabolic network reconstruction. Genes potentially involved in the degradation of phenanthrene were identified based on sequence similarity to genes previously characterized for PAHs degradation.

Conclusions

Five bacterial draft genomes were reconstructed from the metagenome sequences of the TRIP culture. These include two bacterial strains belonging to the *Desulfobacteraceae* family of delta proteobacteria, a putative *Paludibacter*, a *Spirochaeta* and a previously unclassified bacterium. Synteny and phylogenetic studies demonstrated that the members of the *Desulfobacteraceae* family are closely related to the naphthalene-degrading delta proteobacterial strain NaphS2. Two gene clusters encoding the subunits of a carboxylase enzyme potentially involved in the activation of phenanthrene were identified in one of the *Desulfobacteraceae* species (the more abundant operational taxonomic unit of the TRIP culture), suggesting this organism has a major role in phenanthrene degradation.

FEMS7-3003
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ASSIGNING OF FUNCTIONAL ROLES TO INDIVIDUAL BACTERIAL TAXA IN THE CHLORO- AND CHAROPHYTA MICROALGA MICROBIOME

I. Krohn-Molt[1], M. Alawi[2], K.U. Förstner[3], A. Wiegandt[4], L. Burkhardt[5], D. Indenbirken[5], M. Thieß[6], A. Grundhoff[5], J. Kehr[6], A. Tholey[4], W.R. Streit[1]
[1]University of Hamburg, Biocenter Klein Flottbek- Department of Microbiology and Biotechnology, Hamburg, Germany
[2]University Medical Center Hamburg-Eppendorf, Bioinformatics Core, Hamburg, Germany
[3]University of Würzburg, Core Unit Systems Medicine, Würzburg, Germany
[4]Christian-Albrechts-University,
Institute for Experimental Medicine- Division of Systematic Proteome Research & Bioanalytics, Kiel, Germany
[5]Heinrich-Pette-Institute, Leibniz Institute for Experimental Virology- Virus Genomics, Hamburg, Germany
[6]University of Hamburg, Biocenter Klein Flottbek- Molecular Plant Genetics, Hamburg, Germany

Backgrounds

Microalga belonging to the chloro- and charophyta are of high relevance for the global carbon cycling and it is well-known that they are associated with a microbiota. However, it remains unclear, if the associated microbiota is specific for the microalga strains and which role individual bacterial taxa play.

Objectives

Here we provide experimental evidence that chlorophyte (*Chlorella saccharophila, Scenedesmus quadricauda*) and charophyte (*Micrasterias crux-melitensis*) microalga are associated with unique and specific microbial populations.

Methods

Deep metagenome sequencing, binning approaches, secretome analyses in combination with RNA-seq data implied fundamental differences in the gene expression profiles of the microbiota associated with the different microalga.

Conclusions

Our findings indicate that the transcriptionally most active bacteria with respect to key genes commonly involved in plant-microbe interactions in the chlorophyta belong to the phylum of the α-Proteobacteria. In contrast, in the charophyta microbiome bacteria affiliated with the phylum of the Bacteroidetes showed the highest gene expression rates. We furthermore show that effector molecules known from plant-microbe interactions as inducers for the innate immunity are already of relevance at this evolutionary early level.

FEMS7-1364
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

PROFILING AND CONTROLLING THE MICROBIOME OF MICROALGAE IN MASS CULTIVATION PROCESSES

L. Krug[1,2], A. Erlacher[2], T. Cernava[1,3], G. Berg[2]
[1]Austrian Centre of Industrial Biotechnology, Petersgasse 14, Graz, Austria
[2]Institute of Environmental Biotechnology, Petersgasse 12, Graz, Austria
[3]Roombiotic GmbH, Petersgasse 12, Graz, Austria

Backgrounds

Under stressful conditions, the microalga *Haematococcus pluvialis* is capable to accumulate large amounts of the highly valuable carotenoid astaxanthin and is therefore cultivated in large photobioreactors.

Objectives

In mass cultivation, *H. pluvialis* often co-occurs with microorganisms that inhibit and disturb biomass formation and need therefore to be controlled.

Methods

The co-cultivated microbiome was studied in two individually performed mass cultivations by targeting specific taxonomic markers. Co-cultivated microalgae, including *Chlorella*, *Scenedesmus* and *Ochrophyta*, were detected as contaminants already in starting inoculums and could be linked to a substantial reduction of *H. pluvialis* biomass production. In particular, these contaminations accounted for more than 40% of the total eukaryotic biomass in large-scale photobioreactors. By using diazine derivatives we were able to achieve promising results to suppress eukaryotic shares. Illumina HiSeq 16S rRNA gene sequencing revealed a heterogeneous co-occurring prokaryotic community. In two self-contained processes, including reactor upscaling, *Runella*, *Sediminibacterium*, *Sphingomonas* (*Bacteriodetes*) and *Prosthecobacter* (*Verrucomicrobia*) were highly abundant in the first run, while a second analyzed process was dominated by *Comamonadaceae* (*Proteobacter*) and *Flavobacteria* (*Bacteriodetes*). Network analyses revealed reactor-specific cluster formation with 60% unique OTUs. Confocal laser scanning microscopy coupled with LIVE/DEAD staining uncovered a dense biofilm-like colonization behavior within algae aggregates.

Conclusions

The high abundance of co-occurring bacteria potentially positively complements the algae fitness and growth, with antagonistic potential towards unwanted contaminants. Further understanding of the abundant co-occurring microbiome can lead to new biotechnological solutions for contamination prevention and increased biomass yield.

FEMS7-2252
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TOXICITY REDUCTION AND PROTEOMIC ANALYSIS DURING DEGRADATION OF SELECTED XENOESTROGENS BY FILAMENTOUS FUNGUS UMBELOPSIS ISABELLINA.

M. Krupiński[1], *T. Janicki*[1], *J. Długoński*[1]
[1]*University of Lodz, Department of Industrial Microbiology and Biotchnology, Lodz, Poland*

Backgrounds

Numerous pollutants have a harmful effect on the organism through affecting the endocrine system homeostasis. Due to their common use, it is necessary to develop methods for elimination of xenobiotics with endocrine properties from environment. An important compounds of these contaminants are: **4-*t*-octylphenol** (4-*t*-OP), **4-cumylphenol** (4-CP) and **technical nonylphenol** (*t*-NP).

Objectives

The work is a part of research involving identification and characterization of biological systems allowing the reduction of the toxic activity of xenobiotics through their biodegradation. The purpose of the project is acquiring the knowledge about the proteomic background of the xenoestrogens biodegradation (4-*t*-OP, 4-CP, *t*-NP) and obtaining detailed information on the toxic properties of intermediates formed during these processes.

Methods

The assessment of potential toxic properties of biodegradation products was analyzed using bioassays, in which the level of toxicity is tested based on a specific response bioindicators (bacteria, algae, crustaceans, protozoa). Because of the differences in organisms susceptibility to various chemical agents, in this project a battery of tests composed of species representing the various trophic levels of the biological chain were used. In the present work, a proteomic analysis of *U. isabellina* exposed to a tested xenobiotics was conducted using two-dimensional gel electrophoresis (2-DE) followed by MALDI-TOF/TOF identification to reorganize major proteins involved in the biodegradation pathways.

Conclusions

Our results show that the xenobiotics degradation by *U. isabellina* were accompanied by a decrease in the toxicity. Moreover, we observed change in protein expression during incubation of fungus with xenoestrogens, which provides potential evidence for the identification of biodegradation proteomic biomarkers.

FEMS7-2631
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DIVERSE PATTERNS OF BACTERIAL ORGAN FORMATION DURING EMBRYONIC DEVELOPMENT IN LYGAEOID BUGS (HETEROPTERA: LYGAEOIDEA)

S. Kuechler[1], Y. Matsuura[2], R. Koga[3], K. Dettner[1], T. Fukatsu[3]
[1]University of Bayreuth, Animal Ecology II, Bayreuth, Germany
[2]University of the Ryukyus,
Functional Genomics Group- Tropical Biosphere Research Center- COMB, Okinawa, Japan
[3]Bioproduction Research Institute AIST,
Symbiotic Evolution and Biological Functions Research Group, Tsukuba, Japan

Backgrounds

Many hemipteran insects are associated with specific intracellular symbionts housed in symbiotic organs, called bacteriomes. A minor group of true bugs (Heteroptera), especially members of the superfamily Lygaeoidea, also exhibit numerous bacteriome-associated symbiotic systems. The structure and localization of the bacteriomes as well as the symbionts therein offer extensive variations. Overall, six structurally different symbiotic systems have been identified and described within the Lygaeoidea so far.

As described for other intracellular bacteria, endosymbionts of lygaeoid bugs are transferred to the next generation by maternal transmission, usually by integration of the symbionts into eggs during oocyte development. However, detailed processes of symbiont colonization into the developing symbiotic organs have not been studied in most lygaeoid bugs and we almost know nothing about the molecular mechanisms of such an orchestrated phenomenon at the host-symbiont interface.**Objectives**

In order to elucidate bacteriome formation in five lygaeoid species with distinct types of symbionts and symbiotic organs, we analyzed spatio-temporal symbiont dynamics in embryonic development using confocal laser scanning microscopy.

Methods

After collection and fixation of insect embryos at regular time intervals, we traced symbiont relocation and bacteriome development by whole-mount fluorescence *in situ* hybridization (wFISH) in combination with symbiont specific probes.

Conclusions

Comparative analyses of symbiont colonization patterns revealed that symbiont activity as well as the formation of the bacteriomes during embryogenesis are quite diverse in the species corresponding to each symbiotic system characterized in mature insects. Therefore, we propose different developmental mechanisms have independently evolved for bacteriomes of lygaeoid bugs after acquisitions of novel intracellular symbionts.

FEMS7-2057
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

GUT BACTERIAL COMMUNITY OF INDIAN AND FINNISH CHILDREN: INFLUENCE OF FUT2 POLYMORPHISM AND BIRTH MODE

S.V. Kumbhare[1,2], H. Kumar[3], S.P. Chowdhury[1], D.P. Dhotre[1], A. Endo[4], J. Matto[5], A.C. Ouwenhand[6], S. Rautava[3,7], R. Joshi[8], N.P. Patil[9], R.H. Patil[2], E. Isolauri[7], A.R. Bavdekar[8], S. Salminen[3], Y.S. Shouche[1]
[1]National Centre for Cell Science, Microbial Culture Collection, Pune, India
[2]R.C. Patel Arts Science and Commerce College, Department of Microbiology, Shirpur, India
[3]University of Turku, Functional Foods Forum- Faculty of medicine, Turku, Finland
[4]Tokyo University of Agriculture, Department of Food and Cosmetic Science, Hokkaido, Japan
[5]Finnish Red Cross, Finnish Red Cross Blood Service, Helsinki, Finland
[6]DuPont Nutrition & Health, Active Nutrition, Kantvik, Finland
[7]University of Turku, Department of Paediatrics, Turku, Finland
[8]K.E.M. hospital, K.E.M hospital research centre, Pune, India
[9]Smt. Chandibai Himathmal Mansukhani College, Department of Microbiology, Thane, India

Backgrounds

Microbial colonization in the human intestine begins during fetal development which is further influenced by conditions at birth and host genetics. *FUT2* (fucosyl transferase-2) secretor status and birth mode are key determinants of the compositional development of gut microbiota, as documented in western pediatric population. In view of the strong impact of environmental conditions, data from western population may not be valid for different geographical locations; such interactions and effects on the gut microbiota composition in non-western populations remains poorly understood.

Objectives

A comparative study was designed to understand whether alterations of *FUT2* secretor state and birth mode alter gut microbial composition in Finnish and Indian children.

Methods

Blood and fecal samples were collected from 99 children (Age: 13-14 years; Finnish=52, Indian=47) and characterization of the gut bacterial community was carried out using PCR-DGGE and 16S rRNA gene sequencing. Single Nucleotide Polymorphism (SNP) analysis was carried out to determine the *FUT2* SNP rs601338 by genotyping the exonic region of the gene.

Conclusions

The gut microbial composition varied between Finnish and Indian population. *FUT2* secretor state and birth mode had a significant impact upon a number of specific bacterial taxa, patterns of which differed in the two populations. Outcomes of this study highlight the need for exploring and assessing other confounding factors, which may improve our understanding of such interactions/associations in the non-western population, especially in the under-explored and heterogeneous population like India.

FEMS7-2702
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

IDENTIFICATION OF A HEAT STABLE BETA-LACTAMASE, WHICH IS PRODUCED BY BACILLUS CEREUS INACTIVATES PENEM

R. Kuwana[1], R. Yamazawa[1], T. Yamashita[2], K. Honke[2], K. Ito[1], H. Takamatsu[1]
[1]Setsunan University, Pharmaceutical Science, Hirakata, Japan
[2]Kochi University, Center for Innovative and Translational medicine, Kochi, Japan

Backgrounds

Antibiotics are used for the medical care, the domestic animals growth, and the control of health administration. Some antibiotics exposed in the environment are stable and keep the activities, which potentially causes the potential ecological and human health issue. The penem antibiotics, which are hardly to be degraded, are frequently used in Japan.

Objectives

In order to reduce the antibiotics pollution, we aimed to isolate soil bacteria that produce meropenem digestion enzymes.

Methods

We screened *Bacillus* strains that can digest the meropenem from Biological Resource center in Japan and the soils. We found three natural isolates, SUBC1002, SUBC1008, and SUBC1010. We analyzed that they are included in *B. cereus* group.

Conclusions

The MICs of meropenem were 2.5, 3.3, 3.4 mg/l, respectively. We found that the supernatant of *B. cereus* strains after the heat treatment at 80 ℃ was also effective to degrade the meropenem. We resolved the proteins included in the supernatant of *B. cereus* strains by SDS-PAGE and found major two bands with the molecular mass of 28-kDa and 33-kDa bands. We determined their amino acid sequences by LC-MS/MS. The 28-kDa protein corresponded to the *B. cereus* 5/B/6 beta-lactamase II. The 33-kDa protein corresponded to the *B. cereus* 5/B beta-lactamase I. These beta-lactamases belong to the different protein family and highly conserved among *B. cereus* group. These results suggested that some beta-lactamases produced by *B. cereus* strains potentially digested the meropenem.

FEMS7-0802
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

REVEALING OF LANM FUNCTION AND MOLECULAR-GEOECOLOGY IN FUSARIUM WILT PATHOSYSTEM

D.R. Kim[1], S.W. Hong[2], Y.S. Kwak[2]
[1]Gyeongsang National University, Plant Medicine, Jinju, Republic of Korea
[2]Gyeongsang National University, Applied Life Science, Jinju, Republic of Korea

Backgrounds

Crop production is prone to damage inflicted by various plant pathogens. For example, Fusarium wilt which is caused by *Fusarium oxysporum*, is difficult to control because the pathogen produces chlamydospores that survive in soil for many years. *Streptomyces* is a widely-known biocontrol agent that can colonize in the rhizosphere or phyllosphere, which may relate to disease control mechanisms. As a mechanism in the disease suppression system, *Streptomyces* species may act as an agent in specifically suppressing diseases in a long-term crop monoculture system.

Objectives

Fusarium wilt is known to severely affect strawberry cultivation fields and *Streptomyces griseus* S4-7 has been discovered as a representative suppressive strain. The *Streptomyces* secretes diverse forms of antibiotic and other valuable secondary metabolites, but generates only a few lantibiotics.

Methods

Total 7 lantipetide biosynthesis genes in *S. griseus* S4-7 eliminated by homologus knock-out protocol (pKC1132). LanM of *S. griseus* S4-7 showed unique biological properties that differ from other lantibiotics and specific *lanM* gene was detection in soil samples of three locations with qRT-PCR.

Conclusions

Lantibiotics constitute a group of sulfur-peptide with antibiotic properties and heat-stable compounds. Among 7 of lantipeptides, only tsrD, lanA and lanA mutants lost their antifungal activity against Fusarium wilt pathogen. To be suppress soil of strawberry Fusarium wilt takes a minimum of 7 years monoculture, and the *lanM* gene may be used to diagnose whether Fusarium wilt conducive or specific suppressive soil.

FEMS7-0351
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SYMBIOTIC MICROBIOCENOSIS PROTECTION OF INDIVIDUALS AGAINST COMMUNICATING RELATIVELY PATHOGENIC MICROBES ON EXAMPLE OF CANDIDA

V. Lakhtin[1], M. Lakhtin[2], S. Afanasiev[2], A. Bajrakova[2]
[1]G.N. Gabrichevsky research Institute for Epidemiology & Microbiology, Moscow, Russia
[2]G.N. Gabrichevsky research Institute for Epidemiology & Microbiology, Medical Biotechnology, Moscow, Russia

Backgrounds

Human superorganism possesses communicative biotope microbiocenoses. In many cases mixed infections involve eukaryotic pathogens of genera *Candida.* We established principles of communicating microbial “bodies” contracting lectins of human probiotic microorganisms from the same biotopes. Probiotic lectins imitate probiotics and function as metabolomebiotics. The system *Candida-Lactobacillus* characterizes urogenital biotope. Interactions between pools of *Candida* species and *Lactobacillus* species reflect mutual communications.

Objectives

Proposal of the human symbiotic microbiocenosis strategy against pathogenic *Candida*.

Methods

Behavior and reactivity of *C.albicans, C.krusei, C.tropicalis* and *L.acidophilus, L.brevis, L.casei* from intestinal and urogenital biotopes were studied in cultural suspensions in microplate or on agar in the presence of soluble or disc-sorbed symbiotic bacterial lectins (SBL: bifidobacterial and lactobacillar lectins, BL and LL). Biofilms were evaluated using treatment with violet dye followed by its extract detection in visible light region. Leader strains in pools were calculated using our method of ranging biofilms.

Conclusions

1.The following *Candida* species communicative niches criteria were fulfilled. 1.1.Fungi possessed different panels of the utilized carbohydrates. 1.2.Specific landscapes of residual fungal communicative bodies depended on sorbed acidic BL or LL types. 1.3.Opposite and dissonant influences of BL and LL in doses less than 1 mkg/ml towards 48-h-suspension cultures of *Candida* non-albicans were registered. 2.Two identified leader strains of probiotic-like lactobacilli influenced specie-depended distribution of *Candida* on ability to biofilm forming. 3.BL and LL imitated counteractive niches of probiotic bacteria. 4.The data argue the choice and realization of the personal microbiocenosis protective strategy which uses leader symbiotic strains and SBL to alter fungal niches and, as a result, to increase effectiveness of antifungal treatments. Results indicate that SBL participate in antipathogen synergistic mosaic multipointed directed attacks together with leaders as a coupled network of reactions against development of *Candida* infections. Results indicate simplification of prognostic and diagnostic evaluations of communicating niches of yeast like fungi *in vitro*.

FEMS7-1754
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

PROBIOTIC LECTINS: MICROBIOCENOSIS FUNCTIONAL ORGANIZERS

M. Lakhtin[1], V. Lakhtin[1], S. Afanasiev[1], A. Bayrakova[1], V. Aleshkin[1]
[1]G.N. Gabrichevsky research Institute for Epidemiology & Microbiology, Medical Biotechnology, Moscow, Russia

Backgrounds

Communication relationships between microbes in human biotope microbiocenoses are characterized by contribution of many factors. Among them probiotic microorganisms serve the one of key protectors of biotopes. We isolated probiotic lectins (PL, proteins recognizing carbohydrates and their derivatives) and their systems (PLS), characterized preparations of lectins of lactobacilli and bifidobacteria (LL and LB) – cell ingredients of probiotics Acilact, Bifidin, others.

Objectives

Summary of functional features of PLS perspective for effective regulation of human biotope microbiocenoses.

Methods

Intestinal and urogenital *Candida* and *Lactobacillus* species strains were from collection of microorganisms of G.N.Gabrichevsky Research Institute for Epidemiology & Microbiology. Behaviors of microbial cultures (monocultures, *Candida--Lactobacillus* mixtures, microbes in the presence of soluble or disc PL) were studied using microbial cultural suspensions in microplate or on agar. Acidic and alkaline preparations of standardized LB and LL were used. Biofilms were evaluated using violet dye. Leader strains were calculated using comparable ranging method proposed by us.

Conclusions

PL function as PLS which recognize and deliver glycoconjugates (GC such as metabiotics, therapeutics and prebiotics), reveal features of probiotics (antifungal and antibacterial synergism LL and LB to antibiotics and other antimicrobials, imitation of probiotic nishes counteracting the pathogens, cofunctioning to macrophage and to lymphocyte protective systems). PLS function as metabolomebiotics (acting in accordance to the system direction “Probiotic network—Own human network”), a part of Quorum sensing in microbiocenoses and human Cross-Talks involving own protective recognition systems. PLS and GC reveal properties of cofunctioning at the level of biofilm forming (eukaryotic, prokaryotic, mixed; microbial and human; clot-dissolving, destruction and lysis, early and late alterations). Results indicate that PLS are natural multifunctional new key effectors of any human biotope microbiocenosis. The data on structure-function of PLS can serve the guide for diagnostics and prognostics of microbial ecology and microbial communities events; construction of multistrain probiotics, synbiotics and symbiotics.

FEMS7-0658
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ANTIBIOTIC-RESISTANT ESCHERICHIA COLI STRAINS OBTAINED FROM BACTERIA PRESENT IN ORGANICALLY-AMENDED METAL CONTAMINATED SOIL

I. Alkorta[1], C. Garbisu[2], O. Garaiyurrebaso[1], I. Álvarez-Rodríguez[1], F. Blanco[2], I. Martín[2], A. Lanzén[2]
[1]Byophysics Unic UPV/EHU- CSIC, Biochemistry and Molecular Biology, Bilbao, Spain
[2]NEIKER, Conservation of Natural Resources, Derio, Spain

Backgrounds

Organically-amended and metal contaminated soils have been reported as reservoirs of mobile genetic elements (MGEs). Through horizontal gene transfer, MGEs can provide bacteria with a variety of advantageous traits such as, for instance, antibiotic and/or heavy metal resistance. Likewise, resistance to heavy metals and to antibiotics can be associated due to co-resistance or cross-resistance mechanisms.

Objectives

Here, we investigated the presence of plasmids and integrons in metal contaminated mine soil amended with organic wastes, as well as the physiological traits of antibiotic-resistant *Escherichia coli* transconjugants obtained from bacterial communities present in the abovementioned soil.

Methods

Mobilizable IncQ and conjugative IncP-1 plasmids, as well as class 1 integron integrase *intI1* sequences, were detected in soil by PCR-Southern blot hybridization. Through exogenous isolation, antibiotic resistances were captured in *E. coli* transconjugants, which then displayed a multi-resistant phenotype.

Conclusions

Genome sequencing of some of the transconjugants failed to identify novel genes; nonetheless, we identified one missense mutation in the genome of *E. coli* which could be responsible for the observed resistance to aminoglycosides. On the other hand, *E. coli* transconjugants displayed an altered fitness, as reflected by the values of some physiological traits such as growth rate, gyrase activity, RNA:DNA ratio, carbon substrate utilization and biofilm formation. It was concluded that organically-amended metal contaminated soils are a critical reservoir of MGEs harbouring antibiotic resistance genes, with concomitant implications for the dissemination of antibiotic resistance.

FEMS7-1761
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

HIGH-THROUGHPUT SEQUENCING ANALYSIS OF MICROBIAL COMMUNITIES ASSOCIATED TO CARPET-SHELL CLAM

A. Lasa[1], D. Gerpe[1], S. Balboa[1], J.L. Romalde[1]
[1]CIBUS- Facultad de Biología. Universidade de Santiago de Compostela, Microbiology and Parasitology, Santiago de Compostela, Spain

Backgrounds

To date, the studies analysing the bacterial communities associated to bivalves, especially to clams, are focused on the culturable fraction, thus leading to an underestimation of the diversity. The recent introduction of high-throughput sequencing technologies would allow a deeper understanding of the bacterial communities complexity.

Objectives

The present study includes the analysis of the total bacterial community associated to carpet-shell clam (*Ruditapes decussatus*) using 16S rRNA amplicons.

Methods

Samplings of the clams were carried out in summer and winter from two different localities. Different organs (mantle, gills, gonads and hepatopancreas) were analysed separately. The bacterial genomic DNA was obtained and amplified using a set of primers designed for the amplification of the V3/V4 region of 16S rRNA gene. The subsequent amplicons were sequenced at Sistemas Genómicos (Valencia, Spain) using Illumina paired-end sequencing technology.

Conclusions

The analysis of the 16S rRNA amplicons of all the organs taken together and throughout the two samplings, revealed that the major fraction of the sequences belonged to *Proteobacteria*, *Actinobacteria*, *Spirochaetae*, *Bacteroidetes*, *Firmicutes* and *Tenericutes* phyla. Considering all organs, *Proteobacteria* was the most abundant phylum but it showed variations in some organs, being less abundant in the mantle or the hepatopancreas. Bacterial groups that are predominant in the culture-dependent methods, such as *Vibrio* or *Pseudomonas*, were detected but as minor representatives. On the other hand, many genera that are absent using the classic methods were identified including *Caedibacter*, *Endozicomonas* or *Ricketsiella*, together with large groups of uncultured bacteria from different families.

FEMS7-0325
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TOTAL AND POTENTIALLY ACTIVE RHIZOSPHERIC MICROBIOMES OF MELOJO OAK

A.V. Lasa[1], A.J. Fernández-González[1], P.J. Villadas[1], N. Toro[1], M. Fernández-López[1]
[1]Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas, Microbiología del Suelo y Sistemas Simbióticos, Granada, Spain

Backgrounds

Melojo-oak (*Quercus pyrenaica* Willd) is a key tree species of Mediterranean forests with high landscape value in Iberian Peninsula. However, these forests show an advanced stage of degradation and their distribution area has been seriously reduced mainly due to historical human activities and harsh summer conditions typical of Mediterranean regions. In fact, it is regarded as a relict species in Sierra Nevada National and Natural Park. Previous works have suggested that better understanding of plant microbiome, especially of those active microbial members, could be helpful in the development of successful tools for improving plant fitness.

Objectives

The main goal of our work was to study the composition and structure of total and potentially active prokaryotic communities inhabiting melojo-oak rhizosphere, and to identify major bacterial players in order to develop microbiome-based approaches for future afforestation tasks.

Methods

Whole *Q. pyrenaica*'s rhizospheric prokaryotic microbiome and its corresponding active members were studied through 454 pyrosequencing of 16S rDNA and rRNA-derived amplicons. We also calculated the rRNA:rDNA ratio for each core microbiome taxa to get more insights into their potential contribution to the oak rhizosphere environment.

Conclusions

Our results revealed that active bacterial communities are as rich and highly diverse as DNA-based populations. In spite of being composed by nearly the same taxa, the relative abundance patterns differ to a great extent among both studied libraries (rRNA and rDNA). Furthermore, the uncoupling between potential activity and abundance observed for some OTUs showed that the most abundant bacterial taxa are not necessarily the most active.

FEMS7-2054
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

NITRITE DYNAMICS IN THE SEINE RIVER, FRANCE

A. Laverman[1], *T. Cazier*[2], *C. Roose-Amsaleg*[2], *V. Rocher*[3], *M. Raimonet*[2]
[1]*Universite de Rennes 1, UMR 6553 ECOBIO, Rennes, France*
[2]*Universite Pierre et Marie Curie, UMR 7619 Metis, Paris, France*
[3]*SIAAP, Direction du Développement et de la Prospective, Paris, France*

Backgrounds

In the Seine River, France, nitrite concentrations exceed the European norm (0.6µM). Nitrite is produced and eliminated by ammonia oxidizers (AO) and nitrite oxidizers (NO), respectively in the oxic water column.

Objectives

The aim of this study was to get insight in the role of pelagic nitrification in the origin and persistence of elevated nitrite concentrations downstream of Paris.

Methods

To this end, we determined the potential for AO and NO oxidation in the water column and the two main WWTP outlets of the Seine River. In addition to this the key players in ammonia and nitrite oxidation, the ammonia and nitrite oxidizing microbes, were enumerated at the same sites with qPCR.

Conclusions

We showed that the pelagic ammonia and nitrite oxidation potentials were similar whereas nitrite oxidizers outnumbered ammonia oxidizers. The low levels of ammonium in the water column are efficiently oxidized by ammonia oxidizing archeae. However, the relatively low nitrite oxidation rates was in contrast with high numbers of NO. This discrepancy is most likely due to mixotrophic and or heterotrophic growth of *Nitrobacter*, the dominant species in the water column. Rather alternative metabolism of NO than high abundance of pelagic NO in the highly anthropogenic impacted river explains the persistence of nitrite along the Seine River.

FEMS7-1881
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

VIRUSES IN SURFACE WATER AND SEWAGE IN SERBIA

G. Lazic[1], S. Lazic[1], D. Lupulovic[1], P. Knezevic[2], T. Petrovic[1]
[1]Scientific Veterinary Institute "Novi Sad", Department for virology, Novi Sad, Serbia
[2]Faculty of Sciences- University of Novi Sad, Department of Biology and Ecology, Novi Sad, Serbia

Backgrounds

Republic of Serbia does not implement surveillance for the presence of pathogenic human and animal viruses in surface and drinking water, neither is the established methodology of these studies in any institution in Serbia.

Objectives

The aim of the study was detection and molecular characterization of human and animal viruses in surface water and sewage in Vojvodina Province of Serbia.

Methods

The latest methods for concentration of viruses and nucleic acids extraction in surface water and sewage were used in this study. The presence of DNA and RNA viruses was tested by real-time PCR (qPCR) and reverse transcription real-time PCR (RT-qPCR) assays. Molecular characterization was done by Sanger`s sequencing of genome parts of detected viruses.

Conclusions

Human and animal viruses are present in surface water in Serbia. We tested a total of 108 samples of surface and municipal sewage in different seasons, from 2012 to 2014. The analysis of the partial viral genome of detected HAdV from 6 out of 42 positive samples were classified as HAdV group F (types 40 and 41). HEV genotype 3, PAdV type 5 and BPyV type 1 were confirmed in three samples originating from surface water and sewage. The presence of viruses in surface water and of urban sewage reflects the infectious status of the population, and also constitutes a significant risk to the human and animal health in the area that gravitates to that surface water.

Acknowledgments: This work is conducted within the project TR31084 funded by the Serbian Ministry of Education, Science and Technological development.

FEMS7-0681
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COLD STRESS INDUCED BIOFILM FORMATION OF LISTERIA MONOCYTOGENES STRAINS

B. Lee[1,2], *T. Bernardi*[1], *M. Herbraud*[3]
[1]*Biofilm Control, R&D, Saint-Beauzire, France*
[2]*GenXPro, R&D, Frankfurt am Main, Germany*
[3]*INRA, Microbiology, Theix, France*

Backgrounds

Listeria monocytogenes can efficiently survive even in extreme conditions where many other bacteria cannot withstand. Under the assumption that biofilms in food premises leads to food contamination of *L. monocytogenes*, studies have primarily compared the effect of temperature on biofilm formation by growing the bacteria at different temperatures most frequently from 4°C to 37°C and showed that *L. monocytogenes* strains are able to survive at low temperature and form biofilms. However, it is still difficult to directly compare the quantity of biofilms formed at different temperatures since the optimal growth temperature around 37°C involves higher cell growth and enzymatic activities involved in cellular physiology, even though various stress factors including low temperature are known to trigger biofilm formation of *L. monocytogenes*.

Objectives

In this study, we used 22 *L. monocytogenes* strains, of diverse origins and molecular serotypes, to investigate (i) effect of incubation temperatures on cell surface property and its influence on biofilm formation and (ii) effect of cold stress on biofilm formation by comparing biofilms formed upon cold stress and after cold adaptation.

Methods

We applied crystal violet staining to quantify the total biomass and an innovative technique called BioFilm Ring Test® to measure early step of biofilm formation, namely ‘adhesion’ to surfaces. To confirm the biofilm formed at different conditions, we employed scanning electron microscopy.

Conclusions

We confirmed that cold stress enhanced the biofilm formation of all 22 strains. However, there was no correlation between cell surface characteristics and total biomass or adherence of *L. monocytogenes*.

FEMS7-1706
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ANNUAL MONITORING OF GEOSMIN-PRODUCING CYANOBACTERIA AND STREPTOMYCES AND THEIR LINKS TO THE ODOROUS COMPOUNDS IN THE NAKDONG RIVER

C.S. Lee[1], H.S. Oh[2], H.M. Oh[2], C.Y. Ahn[2]
[1]Nakdonggang National Institute of Biological Resources, Bioresources Culture Collection Division, Sangju, Republic of Korea
[2]Korea Research Institute of Bioscience and Biotechnology, Cell Factory Research Centre, Daejeon, Republic of Korea

Backgrounds

Geosmin has an earthy-musty odor and now becoming an important issue in drinking water sources. Both cyanobacteria and actinobacteria were regarded as its microbial source. Levels of geosmin and its seasonal fluctuations have been studied in many freshwater ecosystems, yet there is limited information for the relationships with its microbial sources.

Objectives

The aims of this study was to monitor the concentration of geosmin and to investigate potential relationships between the odorous compound and its microbial sources.

Methods

During April 2014 to March 2015, an odorous compound, geosmin, has been investigated at a water intake tower on the Nakdong River using both the multiplex quantitative PCR(qPCR) and solid phase microextraction(SPME)-gas chromatography(GC). The highest concentration of geosmin was found during June 2014 over 100 ng/L in water, where levels of cyanobacteria and cyanobacterial geosmin-coding gene(*geoA*) were highest as about 40,000 cell/mL and 5,000 copy/mL during the same period. In addition, *Streptomyces*-specific *geoA* gene was also found more than 1,000 copy/mL from the same sample of June 2014. Sequencing analysis of *geoA* genes showed that both *Anabaena* and *Streptomyces* were a major microbial components. However, relatively low amount of geosmin was detected as about 20 ng/L in September 2014, where its microbial source was identified as *Streptomyces.* Statistical analysis indicated that there was a significant relationship between the level of geosmin and cyanobacteria during spring and summer. During autumn and winter, the level of *geoA* and geosmin compound were correlated with the occurrence of *Streptomyces.*

Conclusions

The results suggested both cyanobacteria and actinobacteria could effect on the outbreak of geosmin in the freshwater.

FEMS7-1566
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

NOVEL SPECIES MICROBULBIFER ECHINI ISOLATED FROM THE DIGESTIVE TRACT OF HELIOCIDARIS CRASSISPINA

J.Y. Lee[1], P.S. Kim[1], J.W. Bae[1]
[1]Kyung Hee University, Dept. of Life and Nanopharmaceutical Sciences and Dept. of Biology, SEOUL, Republic of Korea

Backgrounds

The purple sea urchin *Heliocidaris crassispina* is known as algae eater. Algae is composed of specific polysaccharide that difficult to digestive.

Objectives

Investigate commensal bacteria that reside in digestive tract of *H*. *crassispina* is necessary for understanding digestion ability of algae polysaccharide.

Methods

To investigate algae degrading associated specific bacteria in gut of *H*. *crassispina*, we detached gut from *H*. *crassispina* and homogenized it. Bacteria isolation was performed using culture dependent method. Total 110 colonies were selected and were identified by 16S rRNA gene sequencing.

Conclusions

Among them, one bacterium, novel *Microbulbifer* species that was considered as algae associated bacteria was identified. Novel *Microbulbifer* species designated as strain AM134^T belonged to the genus *Microbulbifer* in family *Alteromonadaceae*. Strain AM134^T shared high 16S rRNA gene sequence similarity with *Microbulbifer epialgicus* F-104^T (98.90 % similarity). Strain AM134^T grew optimally at 30 ˚C, in the presence of 2 % (w/v) NaCl and at pH 7. The DNA-DNA hybridization analysis showed the strain shared less than 27 % genomic relatedness with *M. epialgicus* F-104^T. The G+C content of genomic DNA was 56.1 mol%. The major respiratory quinone was identified as ubiquinone-8 (Q-8). The major cellular fatty acids were summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*) and $C_{16:0}$. The results of the phylogenetic, phenotypic and genotypic analyses suggest that strain AM134^T represents a novel species in the genus *Microbulbifer*, for which the name *Microbulbifer echini* is proposed, where the type strain is AM134^T (= KACC 18258^T = JCM 30400^T).

FEMS7-1631
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

KOREAN-DIET RAPIDLY ALTERS THE HUMAN GUT MICROBIOME

N. Lee[1], *M.D. Kim*[1], *E.J. Song*[1], *M.Y. Lim*[1], *Y.D. Nam*[1]
[1]*Korea Food Research Institute, Research Group of Gut Microbiome, Seongnam-si- Gyeonggi-do, Republic of Korea*

Backgrounds

Diet is a major factor driving the composition and metabolism of the intestinal microbiota. We prepared two diets that varied according to their primary food source: a Korean diet, which was rich in cooked rice, kuk (soup), kimchi, side dishes (fermented soy and vegetables); and a Western diet, which was wheat, meat, eggs and fruits.

Objectives

The aim of this study was to investigate the dietary effect of diet composed Korean style (n=5) or Western style (n=5) on gut microbiota composition during 2 week.

Methods

The stool samples were obtained weekly for next-generation sequencing of the 16S rRNA gene (V3-V4 region). The sequences were denoised and further filtered using QIIME software.

Conclusions

Although Western-diet has temporarily increased microbiota diversity during the first week, we observed the reduced microbial diversity between baseline and diet-associated gut microbiota from both diets. To identify the relationship between 15 clinical biomarkers and abundance of microorganism in species level, performed statistical analysis. In Korean- diet, the *Prevotella copri* and *Bacteroides coprophilus* showed the positive correlations with weight change and insulin change, respectively. In Western-diet, the *Bacteroides ovatus* and *Catenibacterium mitsuokai* showed the negative correlation with glucose change. As a result, Korean-diet and Western-diet can alter gut microbial communities in a rapid, diet-specific manner.

FEMS7-0213
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ESTABLISHING A LABORATORY MODEL OF DENTAL UNIT WATERLINE BIOFILMS USING A CDC BIOFILM REACTOR

H.Y. Yoon[1], S.Y. Lee[1]
[1]Gangneung-Wonju National University, College of Dentistry, Gangneung, Republic of Korea

Backgrounds

Water supplied through dental unit waterlines (DUWLs) is often contaminated with bacteria. However, it is challenging to obtain adequate waterline samples for studies of the formation and removal of biofilms.

Objectives

In this study, we established a laboratory model to reproduce DUWL biofilms, using a CDC biofilm reactor (CBR).

Methods

Bacteria obtained from DUWLs were filtered and cultured in R2A liquid medium for 10 days, and were subsequently stored at −70 °C. This stock was cultivated in R2A liquid medium in batch mode. After 5 days of culturing, the bacteria were inoculated into the CBR. Biofilm formation was allowed on polyurethane tubing for 4 days. The accumulation, thickness, morphological characteristics, and distribution of the constituent bacteria of the biofilms were examined by confocal laser scanning microscopy and scanning electron microscopy. The taxonomic diversity of the biofilms was evaluated by pyrosequencing.

Conclusions

Biofilm accumulation on the CBR model was 1.3×10^5 CFU/cm^2 after 4 days. Bacteria were aggregated on part of the surface. Bacteria constituting the biofilms included cocci and rods of short and medium lengths. The thickness of the biofilms after 4 days was 10–14 μm. In addition, 38 bacterial genera were detected in the CBR biofilm. In this study, we demonstrated the suitability and reproducibility of the CBR model for DUWL biofilm formation. The model provides a foundation for the development of bacterial control methods for DUWLs, an important area of dental research.

FEMS7-3170
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SINGLE CELL GENOMIC STUDY OF CANDIDATUS ATRIBACTERIA JS1 LINEAGE PREDOMINANT IN MARINE SEIMENTS OF THE ROSS SEA, ANTARCTICA

Y.M. Lee[1], H. Kuin[1], K. Mincheol[1], H. Soon Gyu[1], S. Seung Chul[2]
[1]Korea Polar Research Institute, Division of Polar Life Sciences, Incheon, Republic of Korea
[2]Korea Polar Research Institute, Unit of Polar Genomics, Incheon, Republic of Korea

Backgrounds

Candidate phylum *Atribacteria* JS1 lineage is among the predominant members in anoxic subseafloor environments, especially in methane-rich sediments. However, the metabolic potential and biogeochemical role of this phylum have remained elusive due to the lack of axenic culture representatives.

Objectives

To understand the metabolic potential and ecological funtion of candidate phylum *Atribacteria* JS1 lineage, single cell genomic approach was applied.

Methods

From the marine sediment at 40 cmbsf of the Ross Sea with 38.9% portion of JS1, 18 single cell amplified genomes considered to be a single species and consistent with the dominant *Atribacteria* JS1 species in that environment were obtained.

Conclusions

The composite genome with the highest genome coverage (2.3Mb) encoded redundant ABC transpoters for amino acid, peptide, and saccharide, complete sets of enzymes in glycolysis and the Wood-Ljungdahl pathway for CO_2 fixation. *Atribacteria* JS1 in the Rose Sea was predicted to be homoacetogenic bacteria, which is capable of growing on H_2/CO_2 or CO_2, and have heterotrophic lifestyle by fermenting various carbon sources. The presence of the Wood-Ljungdahl pathway to run in reverse showed the possibility of *Atribacteria* JS1 as a syntrophic acetate-oxidizer with hydrogenotrophic methanogens which explains their predominance in anoxic methane-rich sediments. Genomic analysis of *Atribacteria* JS1 provided functional potential of this uncultivated but considered to be very important group in carbon cycling.

FEMS7-2555
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ENTEROCOCCUS FAECALIS IN SPANISH WILD AND MIGRATORY BIRDS: FROM POPULATION STRUCTURE TO ACCESSORY GENOME

R. León-Sampedro[1], E. Martins[2], R. del Campo[1], M. Rodríguez-Baños[1], A.P. Tedim[1], M. Pozuelo[1], L. Peixe[2], A. Freitas[2], C. Novais[2], V.F. Lanza[1], F. Baquero[1], T.M. Coque[1]
[1]Insituto Ramón y Cajal de Investigación Sanitaria, Molecular Microbiology, Madrid, Spain
[2]UCIBIO/REQUIMTE - University of Porto, Microbiology Laboratory, Porto, Portugal

Backgrounds

Enterococcus faecalis (*Efs*) is a gut commensal of mammals, reptiles, insects and birds, and one of the main human and animal pathogens able to acquire antibiotic resistance. Sequence information of this species is overrepresented by strains from humans and foodborne animals.

Objectives

To evaluate the role of *Efs* from wild-birds as reservoirs of antibiotic resistance and potential vector of AbR transmission.

Methods

Efs isolates from wild birds (n=100; 9 taxonomic bird orders, two Spanish Centers of Nature Conservation) were analyzed for clonal relationship (PFGE/MLST), and antibiotic susceptibility (CLSI). Virulence and antibiotic resistant genes and mobile genetic elements was inferred by PBRT-PCR, hybridization and sequencing. Comparative genomics (Illumina-HiSeq-2500) of 5 isolates from different bird species was performed to analyze core, accessory genome-(ACCNET) and plasmidome-(PLACNET). The core and accessory genome were compared with available Efs genomes in databases.

Conclusions

Eight major PFGE clusters comprising isolates (n=100) with 41 different ST (25 singletons) detected. Variable resistance to tetracycline (67%-*tet*(M)), chloramphenicol (42%) erythromycin (28%-*erm*(B)) and high-levels of streptomycin (26%), kanamycin (19%) and gentamicin (5%) were observed. Most *Efs* contain 0-3 plasmids (3-90kb) of different families [RepA_N (rep9), Inc18 ($rep1_{pRE25}$; $rep2_{pVEF1}$), Rep_trans-RCR ($rep7_{pS194\text{-}like}$; $rep17_{pRUM}$ and $rep14/orf1_{pEFNP1}$) and Rep3_small_theta ($rep18_{pEF418}$; rep_{pCIZ2}; $rep6_{pAMα1/pS86}$)]. Only MOB_C and MOB_P relaxases were detected. Although the core genome of the sequenced wild bird Efs clusters with ST16, ST55 and ST82 of human and animal origin, a host specific accessory genome (mobile genetic elements, transcriptional regulation and metabolism proteins) was identified. The results highlight the evolvability and networking of *Efs* in different hosts and environments.

FEMS7-0774
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ALTERATION IN GUT MICROBIOME BY DIETARY PROTEIN AND CARBOHYDRATE COMPOSITION IN OBESE VS LEAN DOGS

J. Li[1], C. Lauber[2], G. Czarnecki-Maulden[1], Y. Pan[1], S. Hannah[1]
[1]Nestle Purina PetCare Company, Basic Research, Saint Louis, USA
[2]Nestle Institute of Health Sciences, GI Health & Microbiome, Lausanne, Switzerland

Backgrounds

More than 50% of dogs in the United States are either overweight or obese. Microbial dysbiosis has been associated with metabolic disorders including obesity. High protein low carbohydrate (HPLC) diets have been known for weight management benefits, but their effects on the gut microbiome remains unknown.

Objectives

To evaluate the differential impacts of protein and carbohydrate ratio on the gut microbiome in obese vs. lean dogs.

Methods

Thirty-two Labrador Retrievers and 32 Beagles, half obese or overweight (mean body fat 40%) and half lean or normal (mean body fat 22%) with a mean ages of 5.7, were fed the common baseline diet (phase 1) for 4 weeks, followed by one of the intervention diets (phase 2) for 4 weeks. The HPLC diet contained 49.4% protein and 10.9% carbohydrate, while the low protein high carbohydrate (LPHC) diet had 25.5% protein and 38.8% carbohydrate. Fecal samples were collected at the end of each phase and were subject to 16S rRNA gene sequencing analysis.

Conclusions

Significant diet effects, mostly on the predominant phyla, *Bacteroidetes* and *Firmicutes*, were observed. The effect appears to be greater in obese dogs than in lean dogs, but independent of breed. The HPLC-fed dogs had decreased *Bacteroidetes* to *Firmicutes* ratios, and enriched microbial gene networks associated with weight maintenance, when compared with those on the high carbohydrate diet. The abundances of *C. hiranonis*, *C. perfringens*, and *R. gnavus* were higher in the HPLC group, while those of *B. uniformis* and *C. butyricum* were greater in the LPHC group.

mBio 2017, in press.

FEMS7-2897
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BDELLOVIBRIO PREDATION ON AEROBIC GRANULAR SLUDGE

R. Liébana[1], E. Szabó[1], F. Persson[1], O. Modin[1], M. Hermansson[2], W. Britt-Marie[1]
[1]Chalmers University of Technology, Civil and Environmental Engineering, Gothenburg, Sweden
[2]University of Gothenburg, Department of Chemistry and Molecular Biology, Gothenburg, Sweden

Backgrounds

Predation is one of the main causes of bacteria mortality, having a big influence on the microbial community structure and function. Despite its importance, microbial predation is poorly understood. Wastewater treatment bioreactors offer the opportunity to study basic aspects of microbial ecology, for instance predation, due to the well-defined environment. Predators, such as protists, bacteriophages and predatory bacteria, are considered to have direct impact on the microbial community in bioreactors and hence, on wastewater treatment.

Objectives

The aim was to study the prevalence and predation of *Bdellovibrio* sp. in aerobic granular sludge.

Methods

Cryosections of 9 weeks old granules, harvested from a sequencing batch reactor, were used for fluorescence in situ hybridization (FISH) targeting the *Bdellovibrio* genus (BDE525), ammonia-oxidizing bacteria (AOB; NEU654, Nse1472, Cluster6a192) and total cells (Syto 40). *Bdellovibrio* sp. prevalence was followed in the reactor biomass and the effluent using 16S rRNA gene sequence analysis on the Miseq Illumina platform.

Conclusions

Bdellovibrio sp. was not washed out from the reactors, which indicate that aerobic granules offer a niche to this predator. FISH revealed *Bdellovibrio* sp. actively predating inside the granules, preferentially on AOB, as seen by their localization. This results suggest that AOB, among other bacteria, were subjected to predation which might have an impact on the nitrification process in engineered and natural ecosystems.

FEMS7-0879
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

PREVALENCE OF E. COLI O157:H7 GFP+ ON LEAFY VEGETABLES AS AFFECTED BY PLANT CULTIVAR

J. Lindén[1], T. Bengtsson[1], M. Mieli[2], R. Hartmann[1], L. Mogren[1], M.E. Karlsson[1], B.W. Alsanius[1]
[1]Swedish University of Agricultural Sciences SLU, Biosystems and Technology, Alnarp, Sweden
[2]IUT de Laval, Département Génie Biologique, Laval, France

Backgrounds

Outbreaks of foodborne illness due to consumption of leafy vegetables contaminated with human pathogens have increased during the past 20 years. Interest in maintaining a healthy diet is also increasing and since leafy vegetables are an essential part of a healthy diet the production increases. Several outbreaks have been associated with leafy vegetables contaminated with the harmful *Escherichia coli* O157:H7. Different investigations studied the fate of *E. coli* O157:H7 on the leaf surface and inside the leaf; however, used model crops and cultivars vary widely. Previous studies investigating the impact of the plant cultivar on different plants species have given varying results. We wanted to know if the plant species or the cultivar drives the fate of *E. coli* O157:H7 on leafy vegetables.

Objectives

The plant species selects upon the phyllosphere microbiota and the impact of cultivar of greenhouse grown baby leaf spinach, rocket and Swiss chard is similar.

Methods

A greenhouse experiment was conducted where *E. coli* O157:H7 *gfp*+ was inoculated onto the leaves. The phyllosphere associated bacteria were analysed with viable count, 16S rRNA gene sequencing of pure isolates and metagenomics.

Conclusions

Our results show that the analysed cultivars does not have a significant impact on the colonization of *E. coli* O157:H7 *gfp*+. We demonstrate the phylogenetic diversity of dominating culturable bacteria of the three plant species and three cultivars respectively and show that the phylogenetic diversity however, differ between the plant species and the plant cultivars.

FEMS7-0968
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL DEGRADATION OF AROMATIC FRACTION FROM DIESEL FUEL

M. Ljesević[1], *B. Kekez*[1], *S. Bulatović*[1], *M. Ilić*[2], *G. Gojgić-Cvijović*[3], *V. Beškoski*[1], *M.M. Vrvić*[1]
[1]*University of Belgrade- Faculty of Chemistry, Department of biochemistry, Belgrade, Serbia*
[2]*University of Belgrade- IChTM-Institute of Chemistry- Technology and Metallurgy, Department of Chemistry, Belgrade, Serbia*
[3]*University of Belgrade- IChTM- Institute of Chemistry- Technology and Metallurgy, Department of Chemistry, Belgrade, Serbia*

Backgrounds

Diesel fuel is a complex mixture of alkanes and aromatic compounds that can be harmful if released into the environment. Various microorganisms capable of petroleum degradation were isolated, however the most difficult task is the degradation of polycyclic aromatic hydrocarbons (PAHs). They are a large class of compounds that can be mutagenic and carcinogenic (1). Due to their high molecular masses and hydrophobicity, they adsorb to soil particles, which limits their bioavailability to microorganisms (2).

Objectives

Aim of this study was to separate aromatic fraction from diesel fuel and then to investigate the microbial biodegradation of aromatic fraction.

Methods

Aromatic fraction was separated from diesel fuel on silica gel column by elution with hexane and mixture of hexane:toluene, respectively (3). The bacterial strain *Rhodococcus sp*. RNP05, isolated from petroleum contaminated soil was inoculated into mineral medium where the only source of carbon was aromatic fraction. The remaining aromatic fraction was determined after 30 days by GCxGC/MS.

Conclusions

The aromatic fraction of diesel fuel consists mainly of aromatic hydrocarbons. The results showed the reduction of aromatic fraction concentration in samples after 30 days of degradation. From the GCxGC/MS chromatograms it can be seen that the naphthalens and biphenyls are almost completely removed.

References

1. Chen et al*.*, *Biotechnol. Adv.* 33 (2015) 745-755.
2. Johnsen et al., *Environ. Pollut*. 133 (2005) 71-84.
3. Olsen et al., *Environ. Toxicol. Chem.* 18 (1999) 2448-2453.

FEMS7-0858
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CORRELATION BETWEEN VIRULENCE FACTORS AND QUORUM SENSING SIGNAL MOLECULES IN THREE MARINE VIBRIO PATHOGENS

M. Torres[1,2], J.C. Reina[1], P. Izquierdo[1], I. Llamas[1,2]
[1]University of Granada, Microbiology, Granada, Spain
[2]Institute of Biotechnology. Center for Biomedical Research, Microbiology, Granada, Spain

Backgrounds

The most common and serious bacterial pathogens that affect aquaculture belong to the *Vibrio* species and they are responsible for many diseases such as bleaching in corals and hemorrhagic septicemia (vibriosis) in fish and shellfish.

During recent years the virulence of different pathogenic bacteria has been associated with quorum sensing (QS), a sophisticated mechanism for coordinating gene expression by means of small signal molecules, such as *N*-acylhomoserine lactones (AHLs). In this study, we present the results of AHL inactivation in three aquaculture *Vibrio* pathogens and its correlation with the expression of some virulence factors.

Objectives

To evaluate to what extent the expression of some virulence factors is affected by AHL inactivation in three aquaculture pathogenic strains.

Methods

AHL deficient mutants of *V. mediterranei*, *V. coralliilyticus* and *V. owensii* were constructed by transferring plasmids that contain AHL lactonases and co-cultivation experiments of the three pathogenic species with AHL-degrading strains or QQ enzymes were carried out. The AHL extract in each case was analysed by using AHL biosensor strains. The effect of the AHL degradation upon some of the virulence factors produced by the *Vibrio* species was evaluated by different approaches.

Conclusions

This study shows the correlation between AHLs and the expression of some virulence factors in three aquaculture *Vibrio* pathogens. The information from this research contributes to the development of future new therapies based on AHL disruption, one of the most promising alternatives nowadays for fighting infectious diseases in aquaculture.

FEMS7-2539
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

LITTER DECOMPOSITION IN A WET TROPICAL FOREST: MULTI-TROPHIC IMPACTS ON CARBON TRANSFORMATION AND NUTRIENT MOBILIZATION

E. Lloret Sevilla[1], E.L. Brodie[1], Z. Hao[1], M. Bill[1], J.A. Ceja-Navarro[1], N.J. Bouskill[1]
[1]Lawrence Berkeley National Laboratory, Ecology De, Berkeley, USA

Backgrounds

Phosphorus (P) is an essential yet limiting element within highly weathered tropical forest soils. Leaf litter decomposition likely represents an import source of available P and may be regulated by plant-microbe-metazoan interactions but these mechanisms remain poorly defined leading to uncertainty in models of vegetation dynamics.

Objectives

We initiated a leaf litter decomposition experiment in a humid tropical forest in Luquillo (Puerto Rico) to determine the magnitude of P released from leaf litter decomposition and how this is impacted, by excluding different trophic levels (plant roots, micro and mesofauna, fungi, and bacteria).

Methods

To study decomposition of leaf litter from a dominant tree species (*Dacryodes excelsa*) we collected freshly fallen litter and constructed litter bags with varying mesh sizes to control access to different trophic groups. We deployed these litter bags across two sites with contrasting slopes and redox properties and sampled over 12 months. Bulk chemical analysis of litter C, N and P, together with infrared analysis of leaf chemistry was combined with measurements of biomass across trophic levels.

Conclusions

Preliminary results show lower decomposition rates where access to all trophic levels was possible, although N and P loss was higher. Where roots and fauna were excluded more rapid decomposition of lignocellulose was observed. Ongoing work will determine the microbial players in decomposition and how they are impacted by trophic interactions.

FEMS7-3096
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

HORIZONTAL GENE TRANSFER OF ANTIBIOTIC RESISTANCE GENES IN BACTERIA ISOLATED FROM THE GUT OF THE MUMMICHOG FISH, FUNDULUS HETEROCLITUS

N. Lloyd[1], T. Barkay[2]
[1]Rutgers - The State University of New Jersey, Biochemistry and Microbiology, New Brunswick, USA
[2]Rutgers University, Biochemistry and Microbiology, New Brunswick, USA

Backgrounds

Microbial resistance to antibiotics is a serious public health concern. Antibiotic and metal resistance genes may be linked on mobile genetic elements leading to co-selection whereby resistance to antibiotics is enhanced by exposure to metals.

Objectives

The aim of this research is to investigate co-selection of mercury and antibiotic resistance in a contaminated environment.

Methods

We isolated multi drug resistant bacteria from the gut content of mummichog fish (*Fundulus heteroclitus*) that were collected from Berry's Creek, a legacy mercury contaminated site, and Great Bay, a relatively non-contaminated site in New Jersey, USA. The whole genomes of three strains were sequenced.

Conclusions

Gut isolates were resistant to several antibiotics and mercury and, significantly, mercury resistant strains were more likely to be resistant to 3 or more antibiotics than mercury sensitive isolates ($p < 0.001$). A pattern of co-resistance to ampicillin, chloramphenicol, and trimethoprim was common among isolates representing the *Vibrio*, *Aeromonas*, and *Shewanella* genera. Antibiotic resistance genes were found for all observed phenotypes. Genes encoding for the multi-antimicrobial extrusion (MATE) protein family of drug efflux pumps were found in the isolates. Additionally, the isolates' genomes included genes encoding horizontal gene transfer, such as type IV secretion systems, integrons, transposons, and putative conjugal transfer proteins. Together, the data suggest that a mobile antibiotic resistance gene pool may be created by virtue of a genetic linkage between resistance genes and those that facilitate horizontal gene transfer and microbial adaptation to life in contaminated environments.

FEMS7-1233
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TRANSCRIPTOMIC PROFILING OF A PLANT BENEFICIAL PHOTOTROPHIC BACTERIUM, RHODOPSEUDOMONAS PALUSTRIS PS3 IN RESPONSE TO CHINESE CABBAGE ROOT EXUDATES

K.J. Lo[1], *C.T. Liu*[1]
[1]*Institute of Biotechnology, Institute of Biotechnology, Taipei, Taiwan R.O.C.*

Backgrounds

Rhodopseudomonas palustris strain PS3 is a plant growth promotion rhizobacteria (PGPR) which was isolated from Taiwanese paddy soil. In our previous study, we found that strain PS3 exerted beneficial effects on plant growth by improving efficiency of applied fertilizer nutrients uptake. Moreover, PS3 also reduced the accumulation of nitrate in plant tissue.

Objectives

To explore the mechanisms of plant growth promotion of PS3 and interactions between this bacterium with host plant.

Methods

We performed comparative transcriptomic analyses of PS3 and an ineffective *R. palustris* strain YSC3.in response to root exudates.

Conclusions

Although the genomic backgrounds of PS3 and YSC3 resemble each other, the gene expressions of these two bacteria in response to root exudates were different. Total of 813 and 1,271 genes representing 16.9% and 25.9% of the PS3 and YSC3 transcriptome showed significantly altered expression levels after 24 hours treatment with root exudate. 410 genes (8.5%) and 839 (17.1%) of PS3 and YSC3 were up-regulated after incubation with root exudates for 24 hours, whereas 410 genes (8.5%) and 432 (8.8%) genes were down-regulated. In addition, we also found that several groups of the genes in PS3 which were significantly induced by the root exudates, such as those involved in amino acid metabolism, biofilm formation and flagellar biosynthesis. These results indicate that the interaction between PS3 and host plant plays a critical role in bacterial-stimulated growth promotion. We expect the new insights of this investigation will provide new insights into PGPR, and aid improve efficiency and stability in agricultural practices.

FEMS7-1972
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ORGANIC CARBON AND ORGANIC NITROGEN AT THE CROSSROADS OF FUNGI AND BACTERIA MUTUALISM

A. Lohberger[1,2], J. Spangenberg[3], Y. Ventura[1], N. Jeanneret[1], S. Bindschedler[1], E. Verrecchia[2], R. Bshary[4], P. Junier[1]
[1]Institute of Biology, Laboratory of Microbiology, 2000 Neuchâtel, Switzerland
[2]Institute of Earth Surface Dynamics, Laboratory of Biogeosciences, 1015 Lausanne, Switzerland
[3]Institute of Earth Surface Dynamics, Laboratory of Organic Geochemistry, 1015 Lausanne, Switzerland
[4]Institute of Biology, Laboratory of Eco-ethology, 2000 Neuchâtel, Switzerland

Backgrounds

Roles of organic carbon (Corg) and nitrogen (Norg) as stabilising factors of the interaction between the ectomycorrhizal and saprophytic fungal genus *Morchella* spp. and the soil model bacterium *Pseudomonas putida* were investigated. In *Morchella crassipes*, a complex mutualistic interaction coined bacterial farming, has been previously described. During this interaction three phases were identified: dispersal of bacteria on the fungal mycelium, rearing by the fungus, and harvesting of bacteria. So far, it has been shown that the bacterium benefits during dispersal and rearing, whereas the fungus benefits during harvesting. However, a direct benefit for the fungus during the first two phases remains unidentified.

Objectives

In this study, we further investigated a possible direct benefit for the fungus as a result of bacterial dispersal.

Methods

We tested bacteria moving on the fungal mycelium in a medium containing Corg and Norg, which led to an increase in proteolytic activity by the fungal partner. This increase in proteolytic activity was a general feature of several *Morchella* spp., but required the presence of living bacteria. Comparing the biomasses of both partners as a proxy to fitness, we showed that a truly mutualistic interaction only appears when Corg and Norg are provided in excess. When changing conditions to Corg excess, the fungus took advantage, whereas with Norg without excess Corg, the bacterial partner is favoured.

Conclusions

We conclude that the Corg/Norg ratio is one of the key nutritional factors to determine the outcome of interactions between soil fungi and bacteria.

FEMS7-1946
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

EXPLORATION OF NOVEL DEFENSE STRATEGIES OF BACTERIA USING BACTERIA-PHAGE ANTAGONISTIC COEVOLUTION

A. Lopatina[1], A. Millman[1], E. Abram[1], S. Silverman[1], S. Melamed[1], A. Leavitt[1], R. Sorek[1]
[1]Weizmann Institute of Science, Molecular Genetics, Rehovot, Israel

Backgrounds

Prokaryotes are faced with a constant threat of predation, and have developed a broad range of defense mechanisms against viruses. Recently it has become clear that the whole arsenal of microbial immune systems must be even more complex and diverse, therefore discovery of yet unexplored functional defense systems has attracted more and more attention over the last few years. Experimental approach provides advantages in detection of novel defense strategies, as the memory of host-predator interactions could be recorded in the genomes of bacteria as rearrangements at specific loci, and could be monitored during real-time evolutionary experiments.

Objectives

To discover novel defense strategies of bacteria using bacteria-phage coevolution experiments.

Methods

In this work we performed over 70 separate coevolution experiments with dozens of strains of *Bacillus* and *Thermus* and their phages. Each bacterial strain was infected with one of its phages in 500 µl of liquid media, allowing coevolution for seven days with serial daily transfers. Whole genome sequencing of bacterial and phage genomes was performed, followed by analysis of genome changes suspected as responsible for phage and bacterial survival during the multiple coevolution passages.

Conclusions

Different bacteria-phage growths dynamics were observed during co-incubation, indicating different defense strategies of bacteria. Mutations in phage receptors, lysogenisation and rearrangements in CRISPR arrays were among most common defense strategies of bacteria. In addition, multiple short fragment inversions were detected in the genome of *Thermus* strains after phage infection; further experiments are required to discover the mechanisms of these rearrangements.

FEMS7-0615
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

UNCULTURED AND CANDIDATE LINEAGES IDENTIFIED AS VIABLE MICROORGANISMS IN THE DEEP TERRESTRIAL BIOSPHERE

M. Lopez-Fernandez[1], *D. Simone*[1], *E. Broman*[1], *X. Wu*[1], *S. Bertilsson*[2], *M. Dopson*[1]
[1]*Linnaeus University, Department of Biology and Environmental Science, Kalmar, Sweden*
[2]*Uppsala University,*
Department of Ecology and Genetics- Limnology and Science for Life Laboratory, Uppsala, Sweden

Backgrounds

The 'deep biosphere' is the largest 'bioreactor' on earth and is estimated to contain 2 to 19% of the total biomass. Nevertheless, due to the difficulty of obtaining samples, this milieu is one of the least understood ecosystems on the planet. Microorganisms inhabiting this biome strongly influence the global nutrient and energy cycles.

Objectives

An important question for deep biosphere microbiology is not only its diversity but also whether or not specific populations are viable or not. The Äspö Hard Rock Laboratory provides access to investigate the microbial life in the deep terrestrial subsurface.

Methods

Previous studies at the site revealed the presence of microbial anaerobes, putative H_2 oxidizers and NO_3^-, Fe^{3+}, SO_4^{2-}, and Mn^{2+} reducers along with acetogens and methanogens. The current work expands on these earlier findings to identify the viable (i.e. having an intact cellular membrane) and non-viable (i.e. having a compromised membrane) populations in three aquifers with different chemistries and depth below the surface. High throughput 16S rRNA gene sequencing of total and viable cells revealed significant differences between the subsets of the community and that the viable diversity decreased with temporal separation from the surface. The viable population was mainly related to uncultured candidate phyla such as *Parcubacteria* (OD1), *Gracilibacteria* (GN02), *Atribacteria* (OP9), *Katanobacteria* (WWE3), *Microgenomates* (OP11), Candidate TA06, and unclassified *Euryarchaeota*.

Conclusions

Our results emphasize the importance of unclassified and candidate phyla in the deep biosphere, while ongoing metatranscriptomics analysis will further dissect the metabolic capacities and functions of active microbial populations in this biome.

FEMS7-2186
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COMPARATIVE GENOMICS AND PHYLOGENOMICS OF THE GENUS SALINIVIBRIO

C. Lopez-Hermoso[1], R. Ruiz[1], A. Ventosa[1], C. Sanchez-Porro[1], K. Konstatinidis[2]
[1]Universidad de Sevilla, Microbiology and Parasitology, SEVILLA, Spain
[2]Georgia Tech, Building- Georgia Institute of Technology, Atlanta, USA

Backgrounds

Vibrio costicola was first described by Smith in 1938. This species was then transferred to the genus *Salinivibrio*, which today comprises four species, one of them with three subspecies. Salinivibrios are moderately halophilic bacteria isolated from salted meats, brines and hypersaline environments. Recently, next-generation sequencing (NGS) technology has allowed us to conduct whole genome scan of several members of this genus.

Objectives

The aim of this study was to sequence and to perform a comparative genomic study of 36 strains of the genus *Salinivibrio* including all the type strains of species and subspecies, as well as other representative new strains isolated from different solar salterns in Spain and Puerto Rico.

Methods

WGS was accomplished by using Illumina and PacBio technologies. After assembly, the main features of each *Salinivibrio* genome were studied, their taxonomic affiliation was confirmed by means of phylogenomic analyses and other *in silico* indexes, and the presence/absence of genes, abundance in saline and hypersaline environments and main metabolic pathways were analyzed.

Conclusions

The studied strains can be classified in five different specific lineages. It seems that representatives of *Salinivibrio* are not a predominant population in hypersaline environments despite they can be easily cultivated in artificial media. Genes related to motility, anaerobic respiration, ectoine synthase, choline dehydrogenase, betaine aldehyde dehydrogenase and bacterial type II secretion systems were significantly abundant in the studied genomes.

FEMS7-0739
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

OYSTERS AS RESERVOIRS FOR THE ZOONOTIC SEROVAR OF VIBRIO VULNIFICUS: ROLE OF VVP, VVHA AND RTXA1

C. Lopez-joven[1], C. Murciano[2], J. Perez-larruscain[3], E. Sanjuan[2], A. Llorens[2], C. Amaro[2]
[1]Faculty of Veterinary Sciences, Preventive Veterinary Medicine Institute, Valdivia, Chile
[2]Facultad de Ciencias Biológicas. Universidad de Valencia.,
Departamento de Microbiología y Ecología, Valencia, Spain
[3]Institut de Recerca i Tecnologia Agroalimentàries, IRTA-SCR, Sant Carles de la Ràpita, Spain

Backgrounds

Vibrio vulnificus is a heterogeneous aquatic bacterial species that comprises avirulent and virulent strains responsible for fatal sepsis either by raw seafood ingestion (primary sepsis), or wound infections (secondary sepsis). The species is subdivided into biotypes (Bt), being Bt1 and Bt2 distributed worldwide; Bt1 is the responsible for primary sepsis and Bt2 for fish-handling-associated secondary sepsis. Bt2 is also a zoonotic agent that causes outbreaks of primary sepsis in fish, and it never has been isolated from bivalves.

Objectives

The objective of this work has been to find out if oysters could also be a reservoir for Bt2.

Methods

To this end, we performed a series of colonization experiments in which oysters were infected or co-infected with selected strains of Bt 1 and 2, both of clinical origin, or with the wild-type strain and a derivative mutant (either in *vvp* [protease involved in chemotaxis], *vvhA* [that codifies an unknown hemolysin], or in *rtxA* [toxin involved in invasion, using two polymorphic variants of *rtx*A1; *rtx*$A1_{1,}$ specific for the most virulent Bt1 strains, and *rtx*$A1_3$, specific for Bt2 strains].

Conclusions

The global results suggest that oysters can act as a reservoir for the zoonotic biotype, being both *vvp* and *rtxA* involved in the process. These selected genes are essential for persistence in the oyster probably conferring a higher resistance to the bactericidal effect of the oysters' hemolymph.

FEMS7-1571
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

AN INTEGRATED APPROACH TO UNRAVEL BACTERIOMA OF INDUSTRIAL COMPOSTING

M.J. Lopez[1], *F. Suárez-Estrella*[1], *M.D.M. Jurado*[1], *J.A. López-González*[1], *J. Moreno*[1]
[1]*Universidad de Almería, Dpt. Biologia y Geologia, Almería, Spain*

Backgrounds

Composting is a self-heating process in which organic matter is biotransformed to rich humic-like matter by mesophilic and thermophilic microbial consortia. Most research on composting microbioma focuses on composting processes using specific raw material and technology, and the conclusions obtained are limited to them.

Objectives

The goal of this study was to perform a comprehensive investigation of the structure of the bacterioma in time-series samples of industrial composting of different raw materials while simultaneously evaluating the enzymes produced.

Methods

Samples were collected from 15 industrial composting facilities. Three facilities were sampled for each of five raw materials, namely, municipal solid waste, sewage sludge, vegetal wastes, alperujo (solid waste from olive oil processing) and food processing wastes. In each facility, six samples were collected at critical phases of composting. After DNA extraction the region V3-V4 was amplified. 16S rDNA gene amplicons were sequenced with the Illumina MiSeq platform. Microbial enzymes involved in metabolism of C, N and P were evaluated in samples after aqueous extraction.

Conclusions

The bacterial diversity and succession was strongly influenced by the different operating conditions and raw materials. The enzymes profile showed a pattern marked by a fluctuant hydrolytic activity during the early stages and a dominance of C over N and P metabolism at the maturation. This study enables an unprecedented detailed view and comparison of bacterial community structure, dynamics, and function in full-scale composting operation facilities.

This research has been funded by the Spanish “Ministerio de Economía y Competitividad” Project AGL2015-64512-R.

FEMS7-1459
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CHARACTERIZATION OF CULTURABLE BACTERIAL ENDOPHYTES ASSOCIATED WITH FLOATING MACROPHYTES

M. Saganome[1], A. Delgadillo[1], L. Lozano[1]
[1]Universidad de La Salle, Ciencias Básicas, Bogota, Colombia

Backgrounds

Floating aquatic plants have potential for nitrogen and phosphorus phytoremediation in eutrophic wetlands. Endophytic bacteria inhabit plant tissues without causing any visible negative effect. Endophytes can confer beneficial effects to their host, like tolerance to abiotic stresses and they may contribute to degradation of pollutants. However, there are a few reports on endophytic bacteria associated with floating macrophytes.

Objectives

The aim of this study was to characterize culturable endophytic bacteria isolated from two floating macrophytes (*Lemna sp.* and *Azolla sp*).

Methods

Lemna sp. and *Azolla* sp. were colected at eutrophic wetland in Bogota, Colombia. Endophytic bacteria were isolated from surface-sterilized plants and cultured in Nutrient Agar. Isolates were evaluated in heterotrophic ammonia oxidizers medium and nitrite denitrification medium and bacteria were identified using 16S rRNA gene.

Conclusions

68.8% of isolates were classified as aerobic heterotrophic ammonia oxidizers and aerobic nitrite denitrifiers. Endophytic strains isolated from *Azolla* sp. belonged to phylum Proteobacteria (71.4%), Actinobacteria (14.3%) and Bacteroidetes (14.3%). Strains isolated from *Lemna* sp. were classified into genus belonging to phylum Proteobacteria (67%) and Actinobacteria (33%). *Brevundimonas* was the only genus isolated from both macrophytes. Results suggest that these bacteria endophytes could be candidates for further studies examining their potential for nitrite and ammonium removal in contaminated wetlands.

FEMS7-2164
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TRANSCRIPTOME-SIP OF A HYPOXIC POLLUTANT-DEGRADING AQUIFER MICROBIOME

L. Bradford[1], G. Vestergaard[2], A. Táncsics[3], B. Zhu[1], T. Lueders[1]
[1]Helmholtz Zentrum München, Institute of Groundwater Ecology, Munich Neuherberg, Germany
[2]Helmholtz Zentrum München, Research Unit Comparative Microbioome Analysis, Munich Neuherberg, Germany
[3]Szent István University, Regional University Center of Excellence in Environmental Industry, Gödöllö, Hungary

Backgrounds

Stable isotope probing (SIP) of nucleic acids is a well-established approach in molecular ecology, with most RNA-SIP studies to date focusing on tracing functionally relevant populations in complex microbiota via ^{13}C-labeled 16S rRNA. However, the new mRNA-SIP approach opens the unique possibility to link specific transcripts in complex systems to the metabolism of a particular labelled substrate. This technique has the potential to provide access to a wealth of process-relevant gene expression, without the caveats of current non-target or probe-directed methods.

Objectives

Here, we successfully demonstrate the feasibility of total RNA-SIP for microaerophilic microcosms from BTEX-contaminated aquifer sediments exposed to ^{13}C-labeled toluene. We were specifically interested in the niche partitioning between aerobic and anaerobic catabolic capacities under transitory redox.

Methods

From the total RNA-seq reads (average 30 mio. NextSeq500 reads per sample), an average of 10^5 reads from each sample were identified as transcripts of functional genes (~1-2 %). Gene categories related to cell motility, secondary metabolite formation and xenobiotics degradation were amongst the most highly ^{13}C-labeled transcripts.

Conclusions

Marked ^{13}C-labeling of abundant catechol 2,3-dioxygenase (C2,3-DO) but not of C1,2-DO transcripts were in line with an hypothesis that the first could be especially important in nitrate-respiring, but oxygen-dependent catabolism of aromatics. Uncultured members of the *Rhodocyclaceae* (related to *Quatrionicoccus* spp.) were amongst the most highly abundant and ^{13}C-labelled in total rRNA. this pioneering demonstration of the applicability of transcriptome-SIP to a complex groundwater microbiome reveals the untapped potential of this new targeted approach in environmental transcriptomics.

FEMS7-1266
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CHARACTERIZATION OF A NEWLY HALOPHILIC SALINICOCCUS SP. HMS ISOLATED FROM WADI AN NATRUN, EGYPT

M. Mabrouk[1], H. Al-kordy[1], S. Sabry[2]
[1]Faculty of Science- Damanhour University, Botany and Microbiology Department, Damanhour, Egypt
[2]Faculty of Science- Alexandria University, Botany and Microbiology Department, Alexandria, Egypt

Backgrounds

A wide diversity of organisms exists in soil. Well-adapted groups can be found in extreme environments.Hypersaline ecosystems show a considerably rich diversity of microbes and are biologically very productive. These environments are rich source for novel microbes to be discovered. The wide biotechnological application of these particular microbes is immense and need to be discovered.

Objectives

The aim of this study was to explore, identify a halophilic bacterium from Wadi An Natrun in Egypt and study its characteristics as a potent candidate in biotechnological applications.

Methods

Bacterial isolation

Morphological and physiological characterization

16SrDNA gene sequencing

Determination of extracellular hydrolytic enzymesactivity

Biopolymer and pigment detection

Conclusions

A newly isolated *Salinicoccus* sp. HMS from a soil sample of Wadi An Natrun was characterized.A mucoid, glistening, reddish-orange pigmented colonies on Horikoshi-I agar plates was identified as *Salinicoccus* sp. HMS by morphological, physiological and biochemical characterization and 16S rDNA sequencing. *S.*sp. HMS grew in presence of 0–25% (w/v) of NaCl and at pH 6–11, with optimum growth at 11.7% (w/v) NaCl and pH 9. *S.*sp. HMS participates in halite formation in Horikoshi-I broth supplemented with 2M, 11.7% (w/v) NaCl at pH 8. The bacterium was shown to produce a wide variety of extracellular hydrolytic enzymes including, inulinase, pectinase, amylase, lipase, mannanase, protease, cellulase, and xylanase. The strain was found to be capable to degrade phenol and chlorpyrifos. Moreover, the isolate due to its biopolymer, pigment and enzymes production under extreme condition displays potential biotechnological and bioremediation applications.

FEMS7-1120
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

VIBRIO COMMUNITY PROFILING OF SEA URCHINS AND THE SURRONDING ENVIRONMENT

A. Machado[1,2], *A. Bordalo*[1,2]
[1]*ICBAS-UP - Institute of Biomedical Sciences Abel Salazar- University of Porto, Hidrobiology and Ecology Laboratory, Porto, Portugal*
[2]*CIIMAR/CIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Hidrobiology and Ecology Laboratory, Porto, Portugal*

Backgrounds

Sea urchin is a valuable seafood resource with growing demand because their gonads are considered a gourmet delicacy worldwide. Moreover, they represent an important source of bioactive substances with highly beneficial effects to human health, like polyunsaturated fatty acids and carotenoids. *Vibrio* genus has been associated to disease in sea urchin (wild and aquaculture), and to seafood outbreaks.

Objectives

The aim of this study was to assess the potential pathogenic risk associated with *Vibrio* sp. to both animal survival and fitness, and human consumers.

Methods

The structure and abundance of *Vibrio* sp. community in coastal water and sea urchins (*Paracentrotus lividus*) were evaluated by PCR-DGGE and MPN-PCR approaches. In addition, characterization of vibrio isolates, with special attention to genotypes associate with toxicity and antibiotic resistance, was performed.

Conclusions

Vibrio sp. were successfully detected in all samples, with abundance ranging from 2.46 to 10^4 in water, and up to 10^7 in sea urchin. Significant differences in community profiles between sea urchin-associated and seawater were observed, indicating that diverse and specific groups can thrive within the sea urchin. Of the V*ibrio* species isolated, *V. alginolyticus* clear predominated among the isolates. Nevertheless, *V. parahaemolyticus*, a known leading causative agent of acute gastroenteritis in humans, was detected in the sea urchin gonads in the warmer months (spring and summer). Our results highlight the need to better knowledge about *Vibrio* community dynamics to understand their pathogenic potential and/or environmental importance in the growing demand and culture of sea urchin, and associated food safety context.

FEMS7-2232
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

IMPACT OF IRON AMENDMENT ON SOIL BACTERIAL ABUNDANCE AND DIVERSITY

A. Machado[1,2], R.B.R. Mesquita[1,3], J.F.C. Vieira[3], M. Rangel[4], A.O.S.S. Rangel[3], A.A. Bordalo[1,2]
[1]ICBAS-UP - Institute of Biomedical Sciences Abel Salazar- University of Porto, Hidrobiology and Ecology Laboratory, Porto, Portugal
[2]CIIMAR/CIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Hidrobiology and Ecology Laboratory, Porto, Portugal
[3]Universidade Católica Portuguesa- CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado- Escola Superior de Biotecnologia, ., Porto, Portugal
[4]REQUIMTE-UCIBIO- Instituto de Ciências Biomédicas de Abel Salazar- Universidade do Porto, ., Porto, Portugal

Backgrounds

According to the World Health Organization, two billion people suffer iron deficiency, and the consumption of iron-rich plants can be a way of tackle the problem. Iron has also a crucial role in plant nutrition being an essential element for plant growth, yet one-third of earth soil is iron deficient. The use of synthetic Fe(III)-chelates remains one of the most effective measures to solve this iron deficiency in plants, but their environmental impact must be mastered, since they are highly stable in soils. The search for efficient and sustainable Fe-chelates is, therefore, pressing.

Objectives

The aim of the study was to investigated the effect of iron complexes (synthetic Fe(III)-chelates), on soil bacterial dynamics to better understand their mode of action.

Methods

Soil pots with and without strawberry plants (*Fragaria* sp.), were exposed to different ferrous iron treatments (Fe(III) solution as weak complexes with hydroxide ions, FeEDDHA iron-chelate present in commercially fertilizers, and $Fe(dmpp)_3$ as new alternative iron-chelate). The abundance and diversity of the bacterial community was evaluated by PCR-DGGE and qPCR (*rpoB)* approaches.

Conclusions

No major differences were found in the abundance between soil treatments. Cluster analysis of DGGE profiles revealed that the presence of the plant by itself was not enough to cause a significant change in the bacterial composition. However, the microbial composition seems to shift as a response to Fe(III) amendment and bioavailability. These changes can underline a selection for bacteria that can use Fe(III) in its metabolism, or are more tolerant to its presence, that need to be understood

FEMS7-0873
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

GENOMIC CHARACTERIZATION OF A FRUCTOPHILIC BEE SYMBIONT LACTOBACILLUS KUNKEEI REVEALS ITS NICHE-SPECIFIC ADAPTATION

S. Maeno[1], Y. Tanizawa[2], Y. Kanesaki[3], E. Kubota[3], H. Kumar[4], L. Dicks[5], J. Nakagawa[1], M. Arita[2], A. Endo[1]
[1]Tokyo University of Agriculture, Food and Cosmetic Science, Hokkaido, Japan
[2]National Institute of Genetics, Center for Information Biology, Shizuoka, Japan
[3]Tokyo University of Agriculture, Genome Research Center, Tokyo, Japan
[4]University of Turku, Functional Foods Forum, Turku, Finland
[5]Stellenbosch University, Microbiology, Stellenbosch, South Africa

Backgrounds

Lactobacillus kunkeei is sole obligate fructophilic lactic acid bacterium in the genus *Lactobacillus*, and its unique phenotypic characteristics, including a requirement of electron acceptors for glucose metabolism, are distinct the species from other lactobacilli but are similar to phylogenetically distant *Fructobacillus* spp.

Objectives

The genome sequences of *L. kunkeei* strains were used to study their adaptation to habitats, i.e. fructose-rich niches.

Methods

The genomes of 16 strains of *L. kunkeei* were characterized by comparative genomics against other lactobacilli (n=57) and *Fructobacillus* spp. (n=5). The species possessed significantly less CDSs in smaller genome when compared with other lactobacilli. Functional classification of the genes revealed that *L. kunkeei* had specifically reduced genes for carbohydrate transport and metabolism. The species also lacked most of the genes for respiration, although growth was enhanced in the presence of oxygen. These genomic characteristics were similar to those of *Fructobacillus* spp. The *adhE* gene of *L. kunkeei*, encoding a bifunctional alcohol dehydrogenase (ADH)/aldehyde dehydrogenase (ALDH) protein, lacked the part encoding the ADH domain, which is reported here for the first time in lactic acid bacteria. The deletion resulted in the lack of ADH activity, implying a requirement for electron acceptors in glucose assimilation.

Conclusions

These results clearly indicated that *L. kunkeei* had undergone a specific reductive evolution in order to adapt to fructose-rich environments. The reduction characteristics were similar to those of *Fructobacillus* spp., but distinct from other lactobacilli with small genomes. Fructose-richness thus induced an environment-specific gene reduction in phylogenetically distant microorganisms.

FEMS7-1197
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE EXPRESSION PROFILE OF SECONDARY METABOLITES IN BIOFILMS OF B. AMYLOLIQUEFACIENS CECT 8237 BIOCONTROL STRAIN

M.C. Magno Pérez-Bryan[1], J. Hierrezuelo León[1], A. de Vicente Moreno[2], A. Pérez García[2], D.F. Romero Hinojosa[1]
[1]IHSM-UMA-CSIC. Departamento de Microbiología- Centro de Supercomputación y Bioinnovación- Universidad de Málaga., Microbiology, Málaga, Spain
[2]IHSM-UMA-CSIC. Departamento de Microbiología- Facultad de Ciencias- Universidad de Málaga., Microbiology, Málaga, Spain

Backgrounds

The contribution of *Bacillus amyloliquefaciens* CECT 8237 (UMAF6639) strain to the plant protection against bacterial and fungal pathogens is mainly based on: i) the production of antimicrobial compounds, ii) the plant-growth promotion capability and iii) the induction of systemic resistance in plant host.

In previous works, we demonstrated the relevant implication of the three families of lipopeptides in the biocontrol activity and biofilm formation on melon leaves. The analysis of the genome sequence revealed features previously identified in other *Bacillus* strains, such as genes related to biofilm formation, phytostimulation and induction of systemic resistance in the host plant, and novel genomic regions non-conserved within the *Bacillus* genus, and therefore with potential genes implicated in the biocontrol activity.

Objectives

Considering the relevance of biofilm formation and production of secondary metabolites in biocontrol, we analyzed the expression profile of several secondary metabolites produced by CECT 8237 in biofilm inducing conditions.

Methods

To do so, we optimized an *in situ* detection method based on MALDI-TOF analysis of secondary metabolites within the bacterial colony and in supernatants and pellicles of *B. amyloliquefaciens* biofilms.

Conclusions

We found a major accumulation of these secondary metabolites in the core and middle area of the colony and in the spent medium compared to pellicle.

Further studies will help elucidating the real implication of these molecules in the bacterial ecology or in its mechanisms of defence, against competitors, and/or offense, against pathogens and its possible relation with the niche they occupy.

This work was supported by grants from the Plan Nacional I+D+I (AGL-2012-31968) and Incentivos a Proyectos de Excelencia de la Junta de Andalucía (P10-AGR-57-97), both cofinanced by FEDER funds (EU). This work was also supported by grant from Koppert B.V. (8.06/60.4086) and European Research Council-Starting Grant BacBio ERC637971.

FEMS7-2620
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

RAPID IDENTIFICATION OF VIBRIO SPECIES ISOLATED FROM ACARTIA TONSA COPEPOD EGGS USING MALDI-TOF MASS SPECTROMETRY

M. Zidour[1], M. Chevalier[1], B. Cudennec[1], T. Grard[2], S. Souissi[3], D. Drider[1], C. Flahaut[4]
[1]Institut Charles VIOLLETTE ICV- E.A. 7394- Université Lille 1, 59655, Villeneuve d'ASCQ, France
[2]Institut Charles VIOLLETTE ICV- E.A. 7394-Université du littoral-Côte d'Opale- Boulogne-sur-Mer, 62200, Boulogne-sur-Mer, France
[3]3Univ. Lille- CNRS- Univ. Littoral Cote d'Opale- UMR 8187- LOG- Laboratoire d'Océanologie et de Géosciences, 62930, WIMEREUX, France
[4]Institut Charles VIOLLETTE ICV- E.A. 7394- Université d'Artois- Lens- France., 62300, Lens, France

Backgrounds

Vibriosis caused by *Vibrio spp* infection is one of the most prevalent diseases in the fishes and other aquaculture-reared organisms. Mortality prevention in aquaculture systems by setting-up of preventive or curative adequate treatments is, in part, closely associated with improved methods of pathogenic strains identification.

Objectives

The present study aimed using the MALDI-TOF-mass spectrometry for rapid identification of *Vibrio* species isolated from *Acartia tonsa* eggs issued from the copepod culture collection of the marine station of Wimereux located in the English Channel, northern coast of France.

Methods

From pure colonies grown on the nutrient agar, a sample preparation procedure was established and combined with a rapid and automated MALDI-TOF-MS measurement protocol, performed at least in triplicates. For the bacteria identification, the mass spectra were processed using BioTyper software (version 3.0; Bruker Daltonics) running with the BioTyper database version DB-5989, containing 5,989 reference MALDI-TOF- MS profiles (5,298 of bacteria, 626 of yeasts and 65 of filamentous fungi). Matching between experimental MALDI-TOF- MS profiles obtained from bacteria isolates and the reference MALDI-TOF- MS profiles is expressed by BioTyper according to a Log(Score) and an associated-color code (green, yellow and red). Some identified species were confirmed by DNA extraction, 16S rDNA PCR and sequencing.

Conclusions

The bacteria identification based on MALDI-TOF-MS allowed to fast, easy , cost-effective, and unambiguous identification of *V. anguillarum, V. pomeroyi, V. cyclitrophicus, V. alginolyticus, V. scoophthalmi, V. gigantis and V.changasii*. The more- time consuming bacteria identification method based on the 16S rDNA produced comparable results.

FEMS7-1751
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INSIGHTS INTO TEMPERATURE MODULATED COMMUNITY DYNAMICS OF AN ARSENIC RICH HIMALAYAN GEOTHERMAL SPRING

N.K. Mahato[1], A. Sharma[1], Y. Singh[1], R. Lal[1]
[1]University of Delhi, Department of Zoology, NEW DELHI, India

Backgrounds

Geothermal springs are natural extreme niches where ambient environmental conditions restrict the life only to prokaryotes. Community genomics analyses provide insights into diversity of extremophiles flourishing in these habitats along with their metabolic roles.

Objectives

Metagenomic survey of an arsenic rich Himalayan geothermal spring was performed to elucidate the diversity of microbial communities inhabiting bubbling water and their functional adaptations to withstand high temperature.

Methods

Samples of water were collected from two geothermal spring openings (>96 °C) located at Parvati Valley, Himachal Pradesh, India (32°01′34.8″N, 077°20′50.3″E). Illumina GAII technology was selected for sequencing the community DNA. Taxonomic status was assigned to quality filtered metagenomic raw reads using MetaPhlAn. Functional annotation was done using KAAS and MinPath server. Comparative account of environmental shotgun data from different habitats (bubbling water, sediment and microbial mat) of same geothermal spring was employed to explore habitat specific variations within the same ecosystem.

Conclusions

Diversity analyses revealed predominance of phyla namely, *Proteobacteria, Thermi, Actinobacteria, Crenarchaeota* and *Bacteroidetes* in the water samples. Functional analyses showed genetic enrichment of genes encoding for repair system and homologous recombination highlighting the environment-driven evolution of DNA repair system to withstand high temperature. Additionally, Metagenomic data of water samples was also used to reconstruct draft population genome of *Emticicia* sp. MM along with its four plasmids. Temperature plays a pivotal role in shaping bacterial community and their metabolic adaptations across different habitats of geothermal springs. We observed inter-habitat functional conservedness, however microbial diversity varies at different habitats within same environment.

FEMS7-0094
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CLADOSPORIUM AS AN IMPORTANT ALLERGIC FUNGUS ISOLATED FROM AN EDUCATIONAL AND THERAPEUTIC CENTER, BABOL, IRAN

S. Mahdaviomran Mahdavi Omran[1], F. Nazari[1], M. Hajiahmadi[2], M. Taghizadeh[3]
[1]Babol University of Medical Sciences, Dept. of Medical Parasitology and Mycology, Babol, Iran
[2]Babol University of Medical Sciences, Dept. of Biostatistics, Babol, Iran
[3]Mazandaran University of Medical Sciences, Dept. of Medical Parasitology and Mycology, Sari, Iran

Backgrounds

Fungi are the most important microbial agent to produce of allergy and asthma in the world. Hospitals have a key role in this disorder in hospitalized patients.

Objectives

According to this matter, the present study was performed for isolation and determination of *Cladosporium*, an allergenic fungus in some important wards of an educational and therapeutic center, Babol (Northern Iran).

Methods

The air sampling was performed actively using Anderson's equipment with Sabouraud dextrose agar plate complemented by chloramphenicol (Sc), in two times (morning and afternoon). Totally 63 plates (from 3 important wards) used for survey of *Cladosporium* spp. as an allergenic fungi in the air of an educational and therapeutic Hospital of Babol (Northern Iran). Plates were placed up to 2 weeks in the room temperature; then growth of colonies and the morphology of fungi were examined by light microscope.

Conclusions

In this study 963 colonies were related to *Cladosporium* spp. with 15.29 colony/plates (270.1 colonies/m^3). The most pollution was seen in Internal Care Unit (ICU), which 578 colonies (24.08 colonies/plates, or 425.55 colonies/m^3) were grown on plates. The best ward was the morning surgical room (0.75 colonies/plate or 13.25 colonies/m^3). The most colonies were observed in morning (566 colonies, 15.72 colonies/plates).
The result of this study showed, Cladosporium (an allergenic fungus) is as a prevalent fungus in this Hospital. According to this matter it is necessary to have a suitable planning for decreasing of *Cladosporium* in the air of Hospital.

FEMS7-0602
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INTERACTIONS BETWEEN PHOTOAUTOTROPHIC ORGANISMS AND BELOW-GROUND MICROORGANISMS IN DRYLAND ECOSYSTEMS

S. Maier[1], A. Tamm[1], D. Wu[1], J. Caesar[2], T. Hoffman[3], M. Grube[4], B. Weber[1]
[1]Max Planck Institute for Chemistry, Multiphase Chemistry, Mainz, Germany
[2]University of Applied Sciences, Department of Agriculture and Food Sciences, Neubrandenburg, Germany
[3]University of Cape Town, Department of Botany, Rondebosch, South Africa
[4]University of Graz, Institute of Plant Sciences, Graz, Austria

Backgrounds

Biological soil crusts (biocrusts) play a vital role in dryland regions that cover over 35% of the Earth´s land mass. They provide important ecosystem services such as protection against soil erosion and facilitation of seedling establishment of higher plants. Furthermore, their contribution to the carbon and nitrogen cycle is substantial at a global scale. Cryptogamic covers, including biocrusts, contribute ~7% to the annual carbon uptake by terrestrial vegetation and approximately 40% to the maximum annual terrestrial biological nitrogen fixation.

Objectives

The aim of this study is to test the hypothesis that the successional stage, characterized by a dominating photoautotrophic organism, modifies the local microhabitat and hence influences the composition and physiology of the microbial community.

Methods

We analysed the microbial composition of different successional stages of biocrusts using qPCR and 16S rRNA gene and fungal internal transcribed spacer region sequencing. In a second approach, we studied the physiological response of the different successional stages with regard to their CO_2 gas exchange and nitrogen cycling capabilities under varying environmental conditions.

Conclusions

The 16S and 18S rRNA gene copy numbers as well as alpha diversity values were highest in late successional biocrust stages. We observed that soil respiration rates of late successional biocrusts were approximately seven times higher than those of poorly developed biocrusts, and different successional stages showed distinct nitrogen gas emissions. These findings indicate that organisms within the biocrusts alter the local environment and thereby influence the composition of microbial communities.

FEMS7-2170
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BACTERIA, ARCHAEA AND FUNGI ARE STRONGLY INFLUENCED BY ABIOTIC FACTORS ALONG A 1600 KM ARIDITY GRADIENT

T. Makhalanyane[1]
[1]*University of Pretoria, Department of Genetics, Pretoria, South Africa*

Backgrounds

Climate change is a major threat to biodiversity and functionality across ecosystems globally, resulting in increased desertification. Arid regions, which are generally depauperate, with low vegetation cover and extremely oligotrophic soils are excellent models for understanding the impact of climate change on microbially-derived ecosystem function. However, little is known regarding how increases in aridity may influence the relationship between microbial diversity and ecosystem functionality; specifically, on the contributions of nutrient cycling by microbial guilds at varying aridity levels.

Objectives

Here we assess microbial communities along a west-east aridity gradient across South Africa (sub-humid, semi-arid and arid regions) to determine the environmental effects on microbial community structure and composition.

Methods

We used 16S rRNA gene and ITS sequencing, enzyme activity assays and statistical analysis.

Conclusions

We found significant differences in microbial community composition and structure across the three regions. The fungal class *Agariomycota,* bacterial class *Alphaproteobacteria* and archaeal class *Thaumarchaeota* were abundant across these regions. Soil chemistry had a significant effect in shaping the microbial community composition and structure across the aridity gradient. A high silt content (40 - 60%) was a major determinant of microbial communities, whereas total carbon determined fungal communities structure in sub-humid regions. In addition, β-glucosidase activity occurred within all samples in this region ranging from 60-150 $mmolh^{-1}g^{-1}$ and positively correlated with carbon content (0.4 correlation). In bacterial and archaeal communities, pH was determined to be the influencing factor in arid regions. In summary, abiotic factors influence bacterial, archaeal and fungal community dynamics along an aridity gradient across South Africa.

FEMS7-0559
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BEHAVIOR OF FUNGAL BIOFILMS USED FOR REMEDIATION EXPOSED TO LONG-TERM MICROBIAL STRESS

C. Novotny[1], H. Valkova[2], P. Slosarcikova[2], K. Svobodova[3], K. Malachova[2]
[1]Institute of Microbiology ASCR, Ecology, Prague 4, Czech Republic
[2]University of Ostrava, Department of Biology and Ecology, Ostrava, Czech Republic
[3]Institute of Microbiology ASCR, Laboratory of Environmental Biotechnology, Prague, Czech Republic

Backgrounds

Biofilms of ligninolytic fungi (LF)have potential for remediation of pollutants but their sustainability when competing with other microbes is not well understood.

Objectives

Behavior of mature and nascent biofilms of three LF exposed to long-term microbial stress was compared, extracellular enzyme activities, biodegradation capacity and structural changes of biofilms were measured.

Methods

Shallow-liquid LF cultures immobilized on polyamide were exposed to bacterial/yeast suspensions (10^6 CFU). Fungal growth, CFU, extracellular enzyme activities and degradation of anthraquinone- or azo dyes were determined. SEM and fluorescence microscopy monitored biofilm structure and viability. Activated sludge (AS) was profiled using DGGE analysis of 16SrDNA and EcoPlate assays.

Conclusions

Comparative study of three different LF documented that 90-d exposure of mature fungal biofilms to *Escherichia coli*, *Pseudomonas fluorescens* and *Bacillus licheniformis* bacteria and *Saccharomyces cerevisiae* and *Issatchenkia occidentalis* yeasts did not decrease the fungal degradation capacity. Minor effects of microbial stress on peroxidase and laccase levels were observed. Densely packed biofilms with abundant extracellular polysaccharide were formed even in the presence of competing microbes. Nascent fungal biofilms formed in the presence of *P. fluorescens* but not *B. licheniformis* or *E.coli* lacked high-degradative power. Spontaneous colonization of *P. ostreatus* biofilm by AS selected certain bacterial species as documented by different genotype and phenotype profile. A remarkable ability of mature LF biofilms to resist bacterial and yeast stress was demonstrated.

Acknowledgement: financial support was by QJ1510088, RVO61388971 and LO1208 projects.

FEMS7-3007
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ISOLATION OF MICROORGANISMS FOR BIODEGRADATION OF POLYMER PLASTICS

K. Malachova[1], Z. Rybková[1], J. Malach[2], Č. Novotný[3]
[1]University of Ostrava, Faculty of Science- Biology and Ecology, Ostrava, Czech Republic
[2]University of Ostrava, Pedagogical Faculty, Ostrava, Czech Republic
[3]Institute of Microbiology ASCR, Laboratory of Environmental Biotechnology, Prague, Czech Republic

Backgrounds

Accumulation of recalcitrant plastic polymer waste is a serious environmental problem.

Objectives

Our objective was to isolate bacteria and fungi with a potential to efficiently degrade selected plastic polymers in liquid media cultures.

Methods

Standard enrichment isolation methods employing low-nutrient-concetration media were used to obtain monocolony isolates of bacterial and fungal organisms. Microorganisms were isolated from plastic wastes originating from a polymer production plant, composting sites, anaerobic sludge and soil samples. The ability to degrade plastic polymers was determined in aerobic liquid media at 24 or 28°C using virgin and pretreated [γ (200 KGy)+T (100°C)] LLDPE, LDPE and virgin PP, PS and PVC films during a 2-month degradation. The degradation was measured gravimetrically and with FTIR, TGA, GPC and SEM methods.

Conclusions

A total of 29 bacteria were isolated and identified by 16S rDNA eubacterial primer set. The bacterial community was dominated by *Bacillus* sp. A total of 16 filamentous fungi and 10 yeasts were also identified using 18S and ITS sequencing of SSU rDNA. One bacterial *Bacillus amyloliquefaciens* and three fungal *Trichoderma hamatum, Trichaptum abietinum, Byssochlamys nivea* strains were able to grow on the polymers and attack pretreated and virgin LLDPE and virgin LDPE and PVC. The most efficient strains were *Bacillus amyloliquefaciens* and *Trichoderma hamatum*.

Acknowledgements: The work was supported by projects FP-7 KBBE.2012.3.5-02 BIOCLEAN, EU structural funding Operational Programme Research and Development for Innovation, No. CZ.1.05/2.1.00/19.0388 and National Feasibility Program I, project LO1208 TEWEP.

FEMS7-1717
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL CONSORTIUM WITH DEGRADING, PHYTOPROTECTIVE AND GROWTH-PROMOTING PROPERTIES AS THE BASIS OF COMPLEX BIOPREPARATION FOR SOIL REMEDIATION AND PROMOTION OF CROP HARVESTS

M. Mandryk-Litvinkovich[1], *A. Muratova*[1], *T. Nosonova*[1], *E. Kolomiets*[1]
[1]*Institute of Microbiology - Belarus National Academy of Sciences,*
Laboratory of biological control agents, Minsk, Belarus

Backgrounds

The problem of agricultural land degradation has acquired top priority in latest decades. Anthropogenic activities resulted in soil destructuring, technogenic acidification, pollution with xenobiotics. Agrostress impairs processes in phytocenoses, reduces soil fertility and microbial populations. The most severe impact on soil depreciation is caused by intense pesticide application, abuse of doses of chemical supply leading to their uncontrolled build-up in the environment. A vital factor in alleviating adverse effects of exposure to herbicides is soil recovery utilizing microorganisms possessing a complex decomposing, phytoprotective and growth-stimulating action.

Objectives

The study was aimed at formulation of microbial consortium with elevated plant-protecting and growth-promoting potential capable to break down residual amounts of herbicides of sulfonylurea and imidazolinone series.

Methods

Various investigation methods were used: microbiological, biochemical, molecular-genetical.

Conclusions

An effective consortium comprising 3 native bacterial strains of genera *Bacillus*, *Herbaspirillum* and *Pseudomonas* showing high degrading, phytoprotective and growth-stimulating activities was composed. It is distinguished by the following characteristics:

-suppresses development of phytopathogens from genera *Fusarium, Botrytis, Pseudomonas, Xanthomonas* and contributes to restoration of agrobiocenoses*;*

-drops the level of residual herbicides of sulfonylurea and imidazolinone series in soil (decay extent of herbicides imazamox (50 MPC) and chlorimuron-ethyl (100 MPC) ranges from 45 to 82 % by 7 days;

-eliminates phytotoxic effect of residual herbicide amounts on plants, facilitating 1,2-1,5 times elongation of seedlings and increasing seed germination rate by 25-35%.

The obtained results evidence attractive prospects of introducing microbial constituents into complex biopreparation for soil bioremediation and recultivation.

FEMS7-1915
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BIODIVERSITY AND PRELIMINARY SCREENING OF ENDOPHYTIC FUNGI ASSOCIATED WITH INDIGENOUS MEDICINAL PLANT UMCKALOABA (PELARGONIUM SIDOIDES)

M.C. Manganyi[1], A. Kumar[1], C.C. Bezuidenhout[2], T. Regnier[3], C.N. Ateba[1]
[1]North West University, Biology, Mmabatho, South Africa
[2]North West University, Enviromental Sciences, Mmabatho, South Africa
[3]Tshwane University of Technology, Food and Biotechnology, Pretoria, South Africa

Backgrounds

Medicinal plants well-known to harbour diversity group of endophytic fungi possessing bioactive compounds. Endophytic fungi isolated from marine environments are known to produce secondary metabolites with broad spectrum biological properties including antimicrobial, anticancer, anti-inflammatory and antiviral activities. Despite the fact that the biodiversity of *Pelargonium sidoides* has been established using molecular analysis, this is the first study in which the endophytic fungal species isolated from this plant was assessed for microbial activity.

Objectives

i. isolate and establish the biodiversity of the fungi though phylogenetic assessments;
ii. determine the potential of endophytes in producing bioactive compounds;
iii. assess bioactive compounds with antimicrobial activity

Methods

A total of 133 endophytic fungi were successfully isolated from 50 plants of *Pelargonium sidoides.* The fungal isolates were classified into 32 genera using the internal transcribe spacer (ITS-1 and ITS-4) primers for DNA barcoding as well as translation elongation factor gene (TEF1 or EF1-α gene) for *Fusarium* identification. Identification of the investigating fungal isolates was conducted using morphological and/or molecular techniques. Preliminary screening was conducted by agar diffusion method using an environmental multiple antibiotic resistant *Escherichia coli* strain.

Conclusions

Large proportions (23%) of the isolates were *Penicillium* species while 12% were *Fusarium*. *Alternaria* and *Aspergillus* at 11% respectively representing the biodiversity. Preliminary screening produced growth inhibition zone diameter data of 11mm for *Aspergillus* sp., (KM458796.1), 5 mm for *Penicillium expansum* (LC015096.1), and 4 mm for *Aspergillus niger* (KP172477.1). These findings indicate that endophytic fungi associated with *Pelargonium sidoides* plants possessing bioactive compounds. Further characterisation of the bioactive compounds is being conducted.

FEMS7-1916
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

GRADIENTS IN PROKARIOTIC COMMUNITY AND GEOCHEMISTRY IN AN ALPINE ROCK GLACIER ASSOCIATED POND

I. Mania[1], N. Colombo[2], M. Freppaz[1], M. Martin[1], R. Gorra[1], A. Anesio[3]
[1]University of Turin, DISAFA, Grugliasco To, Italy
[2]University of Turin, Earth Sciences, Torino, Italy
[3]University of Bristol, School of Geographical Sciences, Bristol, United Kingdom

Backgrounds

Rock glaciers (RGs) are geomorphological features widespread in high-elevation alpine environments, consisting of slow-flowing mixtures of rocks and ice, and are considered indicators of ice-rich permafrost presence. Due to their capability to influence waters passing through and originating from them, RGs have the potential to influence connected alpine water bodies, both in terms of water geochemistry and, as pointed out mainly in the last years, overall ecosystem ecology.

Objectives

In this study, we describe archaeal and bacterial communities in the sediments of an alpine RG-associated pond (Col d'Olen Rock Glacier, Valle d'Aosta, Italy), characterized by serpentinitic mineralogy.

Methods

Abundance and diversity of 16S rRNA genes have been assessed by qPCR and MiSeq Illumina sequencing, along a distance gradient from the RG front. We have also analysed the microbial community composition in relationship with different geochemical factors (DOC, TDN, pH, nitrogen forms, major anions and cations), focusing on the sediment-water interface as a major location for biogeochemical cycling and weathering processes.

Conclusions

While community composition shows a certain degree of variation across the gradient, as well as Na^+, Ca^{2+} and Mg^{2+} concentration, increasing with distance from the RG front, microbial abundance and several geochemical parameters (pH, DOC, TDN, NH_4^+ and Si) follow an apparently depth-based distribution pattern. Our results suggest that a complex net of environmental factors, such as water depth, in addition to the environmental gradient derived from icemelt waters, can have a significant impact on the distribution of the prokariotic community and geochemistry along the Col d'Olen Rock Glacier Pond.

FEMS7-2632
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

JOINING THE TEAM: CO-OBLIGATE SYMBIONTS OF APHIDS EVOLVING FROM DISTANTLY RELATED BACTERIA

A. Manzano-Marín[1], A.L. Clamens[1], A. Coeur d'Acier[1], E. Jousselin[1]
[1]Institute National De La Recherche Agronomique INRA,
UMR1062 - Centre de Biologie pour la Gestion des Populations CBGP, Montferrier-sur-Lez, France

Backgrounds

Typically, aphids house the obligate nutritional bacterial symbiont *Buchnera* inside specialised cells called bacteriocytes. *Buchnera* supplies the aphid with essential amino acids and vitamins, thus insuring the correct development of its host. However, some *Buchnera* lineages have lost the ability to fulfil this role, either triggered or rescued by new and younger endosymbionts. One such case are the aphid species from the Lachninae subfamily, where an ancient loss of the riboflavin biosynthetic genes in the genome of *Buchnera* was accompanied by the acquisition of a co-obligate partner.

Objectives

However, co-obligate symbioses are not restricted to this subfamily, and examples of these have been previously reported mainly by microscopic studies. Thus, we sought to discover the identity of these secondary associates, as well as to determine their contribution to the symbiotic consortium.

Methods

Through whole genome sequencing, we have reconstructed the genomes of the co-obligate endosymbionts from several aphid species belonging to different subfamilies, mainly the Lachninae.

Conclusions

We have corroborated that these co-obligate symbionts indeed supplement essential metabolic auxotrophies found in *Buchnera*. Not surprisingly, they have evolved genomes with similar core metabolic capabilities. Also, we have determined that these co-symbionts have evolved from diverse facultative symbiotic taxa associated to aphids as well as free-living bacterial strains. These findings show that co-obligate symbiosis in aphids is more widespread than previously thought. This suggests a fragile mono-symbiotic association between the aphid host and its *Buchnera* symbiont, whose highly degenerated genome could undergo simple metabolic losses leading to a secondary symbiont establishing as a co-obligate one.

FEMS7-2077
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SOIL FORMATION ADJUSTS THE RHIZOSPHERE EFFECT IN STEERING THE BACTERIAL COMMUNITY ASSEMBLY ASSOCIATED TO A PIONEER PLANT

F. Mapelli[1], R. Marasco[2], M. Fusi[2], E. Rolli[1], S. Fodelianakis[2], D. Daffonchio[2], S. Borin[1]
[1]University of Milan, Department of Food- Environmental and Nutritional Sciences, Milan, Italy
[2]King Abdullah University of Science and Technology,
Biological and Environmental Sciences and Engineering Division, Thuwal, Saudi Arabia

Backgrounds

Plants drive the establishment of a specific root microbiome despite the influence exerted by the environment and host genotype. However, it is not clear to which extent plants growing under evolving environmental settings and soil developmental stages maintain the same ability. Chronosequences in polar moraines, representing environmental gradients characterized by increasing soil deglaciation times and different pedogenesis stages, are ideal environments to disentangle the contribution of the rhizosphere effect and the changing soil developmental stages to select the rhizosphere microbiome.

Objectives

We studied the rhizosphere of an autochthonous pioneer plant growing along a High Arctic glacier chronosequence to clarify if the rhizosphere bacterial community assembly is diversely modulated according to the pedogenesis stages.

Methods

16S rRNA Illumina sequencing and OTU co-abundance network analysis were applied to unravel the structure of bacterial communities inhabiting unvegetated and rhizosphere soils. Statistical analyses showed that soil physicochemical parameters and metabolomics traits, characterized by NMR-based analysis of soluble organics, were significantly correlated to microbiome structure in both soil types.

Conclusions

Plant-driven selection was demonstrated independently from the soil developmental stage. Besides a rhizosphere core microbiome, a variable fraction of adaptable bacteria, typical of each pedogenesis stage, was detected. We proved that physicochemical parameters highly relevant for soil formation and fertility drove the assembly of rhizosphere bacterial communities along the chronosequence. Moreover, OTU co-abundance network complexity changed along the chronosequence, showing a different role of the environmental settings in steering the rhizosphere bacterial community assembly, tuned by the soil developmental stage.

FEMS7-2249
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CHANGES IN MICROBIAL DIVERSITY DURING DIFFERENT BLOOM STAGES OF THE TOXIC DINOFLAGELLATE DINOPHYSIS ACUMINATA

I. MARIN[1], M. García-Portela[2], F. Rodriguez[2], B. Reguera[2], J.P. Abad[1]
[1]Universidad Autónoma de Madrid, Biología Molecular, Madrid, Spain
[2]Instituto Español de Oceanografía IEO, Centro Oceanográfico de Vigo, Vigo, Spain

Backgrounds

Dinophysis species of dinoflagellates are world-wide distributed and include photosynthetic organisms producers of lipophilic diarrethic toxins. One of the most frequently found is *D. acuminata*, a strict mixotroph containing plastids (kepto-plastids) that seem to come from the prey dinoflagellates feed on. Laboratory studies have shown that, one of *Dinophysis* preys is the ciliate *Myrionecta*, which itself prey on several cryptophytes.

Objectives

With the aim of determining whether *D, acuminata* can prey in nature on other organisms beside those studied in vitro, we have started a study based on metagenomics to determine the biological diversity, prokaryotic as well as eukaryotic, present along the development of an algal bloom of this species of dinoflagellate.

Methods

Sampling was made in three stages of an algal bloom at Bueu (Pontevedra, Spain) during the period Jan-Jun/2016, corresponding to a stage before the bloom, at the maximum density of dinoflagellate cells, and at the decay phase. Samples were sequentially filtered through polycarbonate 5 and 0.22 µm filters, and DNA extracted. Variable regions V3-V4 of 16S rDNA and 563-1132 of 18S rDNA were amplified by PCR using appropriate primers, and sequenced using MiSeq Illumina technology.

Conclusions

Preliminary bioinformatics analysis of the sequences obtained for 16S rDNA show that the major groups present in peak of the bloom were Alphaproteobacteria (37.3% of the sequences), Bacteroidetes (26.8%), Cyanobacteria (13.9%), Euryarcheota (9.2%), Gammaproteobacteria (7.7%) and Actinobacteria (2.6%). At this point a decrease of Alpha and Gammaproteobacteria and an increase of the other groups were observed from the point of no bloom.

FUNDING:CGl2013-48861-R, MINECO-FEDER-EU

FEMS7-1152
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SIGB AND AGRA REGULATION IN LISTERIA MONOCYTOGENES: EFFECT ON SURVIVAL IN SOIL/RHIZOSPHERE UNDER BIOTIC AND ABIOTIC CONDITIONS

C. Marinho[1,2], D. Garmyn[2], L. Gal[3], C. O'Byrne[1], P. Piveteau[2]
[1]National University of Ireland- Galway, Department of Microbiology, Galway, Ireland
[2]Univ. Bourgogne Franche-Comté, Agroecologie- INRA, Dijon, France
[3]AgroSup- Dijon, Agroecologie- INRA, Dijon, France

Backgrounds

Listeria monocytogenes is the agent of listeriosis, a life-threatening condition in at-risk people. Complex transmission routes between outdoor environments and the food chain result in foodstuff contamination. Sensing of environmental changes can trigger regulation of gene expression, allowing bacteria to adapt their physiology and survive. The Agr cell-cell communication system transcription regulator AgrA is triggered during several environmental conditions including soil, an important reservoir of *L. monocytogenes*. The RNA polymerase σ^B factor aids survival in several stress conditions and may be required for *L. monocytogenes* survival in the soil environment.

Objectives

This study aims to investigate the involvement of AgrA and σ^B in the regulatory network of *L. monocytogenes* during saprophytic life in soil and rhizosphere according to the background biotic environment.

Methods

A collection of in-frame deletion mutant strains (Δ*agrA*, Δσ^B and Δ*agrA*+Δσ^B) was constructed from parental *L. monocytogenes* EGD-e. Strains were inoculated into clay soil mesocosms at different water holding capacities and with or without background microbiota. Kinetics of strains survival was followed during incubation for 14 days. Growth was investigated in the rhizosphere of *Festuca arundinacea* plants *in vitro*. One-week kinetics of strains survival was performed during incubation into climatic chamber.

Conclusions

Depending on the incubation conditions, the fitness of the deletion mutants were affected. During its saprophytic life in soil habitat, *L. monocytogenes* have to cope with ever-changing environmental conditions and adapt in order to sustain life. Integration of various stimuli results in a coordinated response including communication and stress response systems through AgrA- and σ^B-mediated regulation.

FEMS7-2599
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MLST ANALYSIS TO CHARACTERIZE VANA-ENTEROCOCCUS FAECIUM ISOLATES FORM WILD RED-LEGGED PARTRIDGES

V. Silva[1], G. Igrejas[1,2,3], I. Carvalho[4], F. Peixoto[3,5], C. Marinho[3,5], J.E. Pereira[1], R. del Campo[6,7], P. Poeta[2]
[1]University of Trás-os-Montes and Alto Douro, Department of Veterinary Sciences, Vila Real, Portugal
[2]University NOVA of Lisbon, Research Unit on Applied Molecular Biosciences UCIBIO-REQUIMTE, Lisbon, Portugal
[3]University of Trás-os-Montes e Alto Douro, Functional Genomics and Proteomics Unit, Vila Real, Portugal
[4]University of Trás-os-Montes e Alto Douro, Department of Veterinary Sciences, Vila Real, Portugal
[5]University of Trás-os-Montes and Alto Douro, Department of Genetics and Biotechnology, Vila Real, Portugal
[6]University Hospital Ramón y Cajal- and Instituto Ramón y Cajal de Investigación Sanitaria, Microbiology, Madrid, Spain
[7]Red Española de Investigación en Patología Infecciosa, REIPI, Madrid, Spain

Backgrounds

Vancomycin-resistant enterococci (VRE) have been detected in wild animals representing a public health concern. The red-legged partridge is a common game bird and its meat is consumed in several countries including Portugal.

Objectives

This study aims characterize *van*A-*Enterococcus faecium* isolates from Wild Red-Legged Partridges using MLST analysis.

Methods

Three hundred faecal samples of red-legged partridge (*Alectoris rufa)* from the North of Portugal were screened for VRE. Samples were cultured on Slanetz-Bartley agar supplemented with vancomycin (4 mg/L). Enterococcal isolates were tested for antibiotic resistance and virulence genes. Multilocus sequence typing (MLST) was performed to study the genotypic diversity of *van*A-containing VRE.

Conclusions

VRE was recovered from 6 out of the 300 samples corresponding to *E. faecium* species. High values of resistance were detected for vancomycin, erytromycin, ampicillin, tetracycline, teicoplanin, ciprofloxacin and kanamycin and low values for quinupristin-dalfopristin. The *van*A and *erm*(B) genes were detected in all isolates, as well as the *tet*(M) gene in all tetracycline isolates. All 6 isolates harbored the *esp* gene whereas *hyl* gene was detected in 5 isolates. MLST analysis grouped the isolates as ST448 (n=1), ST139 (n=1) and ST18 (n=4). Our findings show that the red-legged partridges could be a reservoir of antimicrobial resistance genes and may contribute to the dissemination and transference of the resistance genes to other animals and humans.

FEMS7-3080
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COUPLING BIOGEOCHEMICAL PROCESS RATES AND METAGENOMIC BLUEPRINTS OF COASTAL BACTERIAL ASSEMBLAGES IN RESPONSE TO ENVIRONMENTAL CHANGES

T. Markussen[1], M. Middelboe[1], V. Kisand[2], E.M. Happel[1], J. Teikari[3], V. Huchaiah[2], J. Alneberg[4], A. Andersson[4], K. Sivonen[3], L. Riemann[5]
[1]Marine biology section, University of Copenhagen, Helsingør, Denmark
[2]Institute of Technology, University of Tartu, Tartu, Estonia
[3]Division of Microbiology and Biotechnology,
Department of Food and Environmental Sciences University of Helsinki, Helsinki, Finland
[4]KTH Royal Institute of Technology Science for Life Laboratory,
School of Biotechnology Division of Gene Technology, Stockholm, Sweden
[5]Marine Biological Section, University of Copenhagen, Helsingør, Denmark

Backgrounds

Marine bacteria are major drivers of biogeochemical nutrient cycles and energy fluxes, yet how they respond to environmental change is not well known. Metagenomic approaches allow examination of genetic responses of the entire microbial community to environmental changes. However, it still remains a major challenge to link molecular information directly to microbial biogeochemical process rates.

Objectives

Here, we investigate metagenomic responses in natural bacterioplankton communities to well-defined environmental stressors in the Baltic Sea, including increased river input, increased nutrient concentrations, and reduced oxygen.

Methods

Using a comparable metagenomics approach, we were able to identify changes in the microbial metagenomic blueprints after exposure to distinct environmental conditions, and to link these changes to biogeochemical process rates and microbial activity.

Conclusions

The results provide new knowledge for developing models of ecosystem structure and biogeochemical cycling in future climate change scenarios and advance our exploration of the potential use of marine microorganisms as markers of monitoring environmental conditions.

FEMS7-2331
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

NEW MARINE GROUP III EURYARCHAEOTA, FROM DARK TO LIGHT

A.B. Martin Cuadrado[1], *J.M. Haro-Moreno*[2], *I. Garcia-Heredia*[1], *F. Rodriguez-Valera*[2]
[1]*Universidad de Alicante, Departamento de Fisiología- Genética y Microbiología, Alicante, Spain*
[2]*Universidad Miguel Hernández, Departamento de Producción Vegetal y Microbiología, Alicante, Spain*

Backgrounds

Marine Euryarchaeota remain among the least understood major components of marine microbial communities. Marine group II Euryarchaeota (MG-II) are more abundant in surface waters (4–20% of the total prokaryotic community), whereas marine group III Euryarchaeota (MG-III) are generally considered low-abundance members of deep mesopelagic and bathypelagic communities.

Objectives

Up to date, there are no cultivated representatives and little is known about their physiology and ecological role in the oceans. Therefore, to obtain representative genomic sequences will help in this sense.

Methods

Using genome assembly from direct metagenome reads and metagenomic fosmid clones, we have identified six novel MG-III genome sequence bins from the photic zone (Epi1–6) and two novel bins from deep-sea samples (Bathy1–2).

Conclusions

Photic-zone MG-III bins corresponded to novel groups with no similarity, and significantly lower GC-content when compared with previously described deep-MG-III genome bins. As found in many other epipelagic microorganisms, photic-zone MG-III bins contained numerous photolyase and rhodopsin genes, as well as genes for peptide and lipid uptake and degradation, suggesting a photoheterotrophic lifestyle. Phylogenetic analysis of these photolyases and rhodopsins as well as their genomic context suggests that these genes are of bacterial origin, supporting the hypothesis of an MG-III ancestor that lived in the dark ocean. Epipelagic MG-III occur sporadically and in relatively small proportions in marine plankton, representing only up to 0.6% of the total microbial community reads in metagenomes. Most low-GC bins were highly enriched at the deep chlorophyll maximum zones, with the exception of Epi1, which appeared evenly distributed throughout the photic zone worldwide.

FEMS7-2021
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DISENTANGLING MECHANISMS INVOLVED IN THE ADAPTATION OF MICROCYSTIS AERUGINOSA TO THE EXTREME SULPHUREOUS WATER FROM LOS BANOS DE LA HEDIONDA (S SPAIN)

E. Martín-Clemente[1], I.J. Melero-Jiménez[1], A. Reul[2], M. Hernández-López[3], E. Salvo[1], E. Bañares-España[1], M.J. García-Sánchez[1], A. Flores-Moya[1]
[1]Facultad de Ciencias- Universidad de Málaga, Biología Vegetal, Málaga, Spain
[2]Facultad de Ciencias- Universidad de Málaga, Ecología y Geología, Málaga, Spain
[3]Facultad de Ciencias- Universidad de Málaga, Química Analítica, Málaga, Spain

Backgrounds

Los Baños de la Hedionda (Málaga, S Spain) is a natural sulphureous spa where sulphide can reach a concentration of 150-200 µM. Although this ion has biocide properties, including inhibition of the photosynthetic process, a rich flora can be found in this extreme environment.

Objectives

To study the adaptation mechanisms allowing resistance of photosynthetic microorganisms to these sulphureous waters

Methods

For this purpose, a modified Luria–Delbrück fluctuation analysis was carried out. The adaptation to La Hedionda waters of three different strains of the cyanobacterium *Microcystis aeruginosa* (Kützing) Kützing (isolated from a non-sulphureous freshwater reservoir) was analyzed in order to find out if it was achieved by a physiological adaptation process (acclimation) or by the selection of rare spontaneous mutations (genetic adaptation).

Conclusions

Several resistant strains were obtained after 6 weeks of cultivation with La Hedionda waters. The fluctuation analysis showed that genetic adaptation was the phenomenon that allowed resistant *M. aeruginosa* cells from the three strains to survive, with similar mutation rates in the order of magnitude of 1 mutant resistant cell per 10^6-10^7 cell division^{-1}. It could be hypothesized that this cyanobacterium could adapt to sulphureous environment by the selection of favoured mutants.

FEMS7-2105
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CHARACTERIZATION OF OSCILLATORIA SP. ISOLATED FROM EXTREME SULPHUREOUS WATER FROM LOS BANOS DE LA HEDIONDA (S SPAIN)

E. Martín-Clemente[1], I.J. Melero-Jiménez[1], A. Reul[2], M. Hernández-López[3], E. Bañares-España[1], A. Flores-Moya[1], M.J. García-Sánchez[1]
[1]Facultad de Ciencias- Universidad de Málaga, Biología Vegetal, Málaga, Spain
[2]Facultad de Ciencias- Universidad de Málaga, Ecología y Geología, Málaga, Spain
[3]Facultad de Ciencias- Universidad de Málaga, Química Analítica, Málaga, Spain

Backgrounds

Los Baños de la Hedionda (Málaga, S Spain) is a natural sulphureous spa (150-200 µM sulphide). Although this high sulphide levels can affect the photosynthetic process, there are numerous photosynthetic microorganisms inhabiting the spa. Among them, we isolated a strain of the cyanobacterium *Oscillatoria* sp., a genus well known by its tolerance to sulphide.

Objectives

Firstly, to analyze the photosynthetic characteristics and growth rate of the isolated strain, as well as the effect of the presence of sulphide in both processes. Secondly, to determine the limit of genetic adaptation of this strain to sulphide.

Methods

The resistance of the isolated strain to sulphide was studied by analyzing the effect of increasing sulphide levels (up to 1600 µM) on photosynthetic performance and growth. The limit of genetic adaptation was explored using an evolutionary experimental design named as ratchet protocol. This design allows discerning the maximum capacity of genetic adaptation of *Oscillatoria* sp. to the exposure of increasing doses of sulphide

Conclusions

The strain showed maximum growth rates at 200 µM sulphide although reduced rates can be found up to 800 µM sulphide. A significant increase in resistance was achieved in all derived populations during the ratchet experiment (surviving at sulphide concentrations higher than 2 mM). Moreover, they showed different evolutionary potential to adapt to sulphide, depending on historical contingency.

FEMS7-1301
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROCOSMS FOR EVALUATING MICROBIAL INDICATOR PERSISTENCE AND MOBILISATION IN FLUVIAL SEDIMENTS DURING RAINFALL EVENTS

J. Martín-Díaz[1], C. García-Aljaro[1], M. Pascual-Benito[1], W. Calero-Cáceres[1], B. Galofré[2], A.R. Blanch[1], F. Lucena[1]
[1]University of Barcelona, Department of Genetics- Microbiology and Statistics, Barcelona, Spain
[2]Aigües de Barcelona EMGCIA, -, Barcelona, Spain

Backgrounds

Climate change and demography evolution would probably increase the intensity and frequency of heat waves and extreme precipitations in several places in the Earth. The study of Mediterranean rivers, subjected to long dry periods, heavy rainfall events and punctual severe droughts could be useful to understand future climate scenarios. Riverbank sediment resuspension into the water column due to heavy rainfalls entails pathogen mobilisation that causes the water quality decline, with the consequent health risk for humans and animals and also the environment deterioration.

Objectives

The evaluation of indicator persistence (T_{90}) and mobilisation from sediments into the river water during the simulation of rainfalls events in 42-day-duration microcosms.

Methods

Considering the One Health concept, six microbial indicators were evaluated in microcosms simulating rainfall events naturally occurring in a Mediterranean river (NE Spain). Flow, turbidity and *E. coli* data from the river were used to set microcosms conditions. T_{90} in sediments were calculated and mobilisation patterns were studied.

Conclusions

T_{90} obtained in sediments were: sorbitol-fermenting *Bifidobacterium*, 4 days; qPCR-detected *E. coli*, 11 days; bacteriophages infecting *Bacteroides thetaiotaomicron* strain GA17, 36 days; culturable *E. coli*, somatic coliphages and spores of sulfite-reducing clostridia, more than 42 days. Different mobilisation patterns were found for bacteriophages and bacteria. Five out of the six indicators were still detected in sediments and water after 42 days, warning against the health risk of pathogen mobilisation into the water column. Valuable data were generated for enhance predictive models that evaluate the effects of climate change over surface waters quality.

FEMS7-0537
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

METAL BIOREMEDIATION CAPACITY OF TETRAHYMENA THERMOPHILA ADAPTED STRAINS TO EXTREME METAL CONCENTRATIONS

P. De Francisco[1], J.C. Gutierrez[1], A. Martin-gonzalez[1]
[1]Universidad Complutense De Madrid, Microbiologia-III, Madrid, Spain

Backgrounds

Heavy metals are considered one of the most toxic and soil persistent pollutants. Due to several antropogenic activities, their environmental levels have increased in the last years and they can cause serious health problems. Physical-chemical techniques or phytoremediation try to remove metals from the environment but they are not so effective in many cases and new tools for metal bioremediation are required.

Objectives

The aim of this study was to analyze the metal bioremediation capacity of three metal-adapted strains from the ciliate *Tetrahymena thermophila.*

Methods

Metal adapted strains were obtained after exposing cells over time to increasing metal (Cd^{2+}, Cu^{2+} or Pb^{2+}) concentrations. They were characterized by TEM and fluorescence microscopy and their bioremediation capacity was studied by flow cytometry and ICP-AES.

Conclusions

The maximum tolerated concentrations (MTCs) of these metal-adapted strains were: 115 mM Cd^{2+} for Cd-adapted strain, 4 mM Cu^{2+} for Cu-adapted strain and 5.5 mM Pb^{2+} for Pb-adapted strain, being these values 2.5, 13 and 6-fold the LC_{50} of the control strain, respectively. Metal toxicity was analyzed by bioassays using control cultures after metal removing by adapted-strains and we observed a significant cell mortality reduction in all cases. Likewise, Pb-adapted strain removed about 95 or 99% of metal after only 24 or 48h exposures, respectively. In our opinion, these ciliate metal-adapted strains could be good cellular tools for metal bioremediation in both soil and aquatic ecosystems.

FEMS7-1025
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ROLE OF COPPER IN PREDATION BY MYXOCOCCUS XANTHUS

G. Martínez Navajas[1], *J. Pérez*[1], *F.J. Marcos-Torres*[1], *A. Moraleda-Muñoz*[1], *J. Muñoz-Dorado*[1]
[1]*Facultad de Ciencias. Universidad de Granada., Microbiología, Granada, Spain*

Backgrounds

Predation is a strategy that is present in multiple living beings. Among the eukaryotes, some organisms are able to prey, such as the amoebas or the macrophages. These organisms use the oxidative stress generated by the presence of divalent metals, among others strategies, for lysing other microorganisms.

M. xanthus is a social bacterium able to prey on other microorganisms. In this bacterium 12 systems that are inducible by the presence of metals in the medium (mainly copper) have been identified. They participate in the oxidation and the extrusion of metallic cations to the exterior. However, these systems do not protect this bacteria from high metal concentrations, so they could have a different function as it could be participating in bacterial predation, in a similar way as the eukaryotic organisms do.

Objectives

For this reason, we have tried to determine whether these systems participate in bacterial predation.

Methods

We have constructed 12 strains harboring fusions between genes of each system and *lacZ*, and analyzed the expression of each system during predation of *M. xanthus* on *Sinorhizobium meliloti* on agar plates containing X-gal and different metal concentrations.

Conclusions

We have observed that some systems are induced in the interface between predator and prey, indicating that at least copper accumulates in this area. It remains to be elucidate whether metals are used as weapons by the predator or as a mechanism of defense by the prey.

This work has been supported by the Spanish Government (grant BFU2016-75425-P).

FEMS7-2431
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DIETS AND ENTEROCOCCI, A MURINE MODEL STUDY

M. Martínez-Cañamero[1], B. Sánchez[1], A. Cobo[1], M. Hidalgo[1], I. Prieto[1], N. Martínez[1], A. Gálvez[1]
[1]Universidad de Jaén, Ciencias de la Salud, Jaén, Spain

Backgrounds

The genus *Enterococcus,* part of the lactic acid bacteria (LAB), is of great importance in food because of its involvement both in fermentations and their deterioration. However, it is also an important nosocomial pathogen with strains with virulence traits and resistance to antibiotics.

Objectives

During our studies on the influence of different diets on the intestinal microbiota, we isolated several strain collections from mice faeces and detected a strain, framed in the species *E. faecalis*, of special interest in the host. We set out to further study this relation.

Methods

The enterococci were physiologically characterized, searched for virulence traits, bacteriocin production, as well as genotyped by Randomly Amplified Polymorphic DNA (RAPD). The strain of interest was used in an intervention study conducted on ten inoculated mice, compared to ten other mice treated with placebo. After three weeks, physiological variables were measured in mice as well as enterococci were isolated in stools, in order to know which animals had acquired our strain, as measured by RAPD. The strain was further studied by Multilocus Sequence Typing and, together with a second one isolated from faeces from mice that did not acquire the inoculum, it has been characterized at the proteomic level by two-dimensional SDS electrophoresis and by mass spectrometry.

Conclusions

Results will be shown in order to give a global picture about the presence of intestinal enterococci in a murine model, on how they are influenced by the diet of the host and, in their turn, how they affect their hosts.

FEMS7-0197
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

FITNESS ANALYSIS OF TETRACYCLINE RESISTANT AND SENSITIVE SHIGELLA FLEXNERI IN THAMES RIVER WATER MICROCOSMS UNDER SUB-LETHAL CONCENTRATIONS OF TETRACYCLINE.

R. Maruzani[1], M. Marvarsi[1], A. Shah[1], V. Serafim[1]
[1]Middlesex University, Natural Sciences, London, United Kingdom

Backgrounds

It is becoming increasingly important to monitor how antibiotics influence fitness of microbial populations in the environment due to emergence of antibiotic resistance.

Objectives

We analyse the fitness of resistant and sensitive *S. flexneri* strains under tetracycline selection pressures in Thames River water environments.

Methods

Microcosms of Thames water from upstream, central London region and downstream inoculated with zero, lethal and sub-lethal concentrations of tetracycline were prepared. Approximately 10^4 CFU/mL isogenic pair of *Shigella flexneri* 1100 (resistant) and 1363 (sensitive) were added to each microcosm at a 1:1 ratio. Fitness was measured by comparing resistant and sensitive colonies after two days' exposure to selection pressure. PAH concentration and tetracycline degradation was determined using UPLC with fluorescence detection and HPLC with tandem mass spectrometric detection, respectively.

Conclusions

S. flexneri 1363 was found to significantly outcompete 1100 at sub-lethal and lethal concentrations of tetracycline in downstream microcosms, where PAH levels were highest. Upstream, *S. flexneri* 1100 outcompeted the sensitive strain in the same environment. The concentration of benzo(a)pyrene in upstream, London and downstream samples was found to be 0.24µg/mL, 1.35µg/mL and 30.79 µg/mL, respectively. Phenanthrene was detected at 1.10 mg/L, 20.51 mg/L and 140.90 mg/L in upstream, London and downstream sites respectively. Antibiotic degradation was observed in downstream lethal samples from 25 µg/mL to 10 µg/mL over 5 days.

Our results suggest PAHs in the environment may complex with tetracycline rendering it bio-unavailable. Resistance genes become a selective disadvantage where tetracycline is not bioavailable. Degradation of tetracycline may also contribute to selection of sensitive strains.

FEMS7-1805
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBORER COLONIZATION OF A SCLERACTINIAN CORAL HOLOBIONT, ACROSS ITS LIFE STAGES

A. Masse[1], A. Tribollet[2], I. Domart-Coulon[1], S. Golubic[3]
[1]Museum National d'Histoire Naturel, UMR7245, Paris, France
[2]Institut de Recherche pour le Développement, UMR7159, Paris, France
[3]Biological Science Center, Boston University, Boston, USA

Backgrounds

Although filamentous microborers are known as one of the major agents of carbonate dissolution in coral reefs, their diversity and dynamics of colonization in early life stages of reef-building scleractinian corals remains unexplored.

Objectives

Here, the microboring Ulvophyceae *Ostreobium* sp. was detected in the skeleton of the branching coral *Pocillopora damicornis* (type beta lineage), from the primary polyp to the budding juvenile colony and in adults.

Methods

Larvae of the *P. damicornis* were allowed to recruit and develop for 3 months on carbonate or non-carbonate substrates, both initially colonized by natural biofilms in aquarium settingsDiversity of *Ostreobium* in adult coral colonies was compared between three aquaria and two reefs sites. Morphological criteria were confirmed by phylogenetic analysis of *Ostreobium rbc*L chloroplastic marker, and distribution and abundance of microborers were quantified using thin sections.

Conclusions

Surprisingly our results show that 7 days old juveniles (primary polyp stage) were already colonized by microborers. Their abundance depended however on the nature of the settlement substrate and its degree of pre-colonization by microborers. The coral growth rate also affected the ability of microborers to reach the skeletal interface with tissue resulting in a ‘dilution effect’ of microboring filaments towards the apex of juveniles (1-3 months-old) and adult coral colonies. Altogether, 8 *rbc*L clades of *Ostreobium* were detected in the studied holobiont, with one dominant clade shared by all life stages, and also present in seawater, carbonate settlement substrates, and reef settings. These results suggest that in living corals the geography influences *Ostreobium* clade distribution and dominance.

FEMS7-0547
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

METSCHNIKOWIA PULCHERRIMA INFLUENCE ON FILAMENTOUS FUNGI METABOLISM.

J.J. Mateo Tolosa[1], *S. Maicas*[1], *R. Ferragut*[1], *Z. Raquel*[1]
[1]*Universitat de Valencia, Microbiología y Ecología, Valencia- Burjassot, Spain*

Backgrounds

Yeasts have the ability to survive in variable environmental conditions without the production of toxic compounds that could affect the human health. Moreover, yeasts can be produced in fermenters on cheap media and are able to survive in a wide range of environmental conditions. Competition with yeasts can dramatically inhibit the growth of fungi such as *Aspergillus, Botrytis, Penicilium* and *Alternaria* by affecting their secondary metabolism.

Objectives

The aim of this work was to determine de influence of *Metschnikowia pulcherrima* simultaneous growth with development of different interesting molds

Methods

M. pulcherrima yeasts were isolated from grapes surface and identified following both physiological and molecular methods. So, they were analysed by RFLP analysis of the 5.8S-ITS rDNA region and for sequence analysis of the D1/D2 domains of the 26S rDNA gene, PCR amplification was performed. Interactions between yeast and mold development were determined by simultaneous inoculation and growth on Malta Agar plates. Inhibition degrees were determined by using ImageJ software.

Conclusions

Once *M. pulcherrima* isolates were positively identified, all studied isolates have shown a high capability to inhibit mold growth and also to interfere their secondary metabolism. These results could open a new door in the field of biological control of fungal contamination, avoiding economical loses produces by this fact.

This work was supported by the project “Identification and biotechnological characterization of yeasts isolated from agrifood residues of the Valencian Community”, grant AICO-2016-079.

FEMS7-0565
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MOLECULAR ANALYSIS OF VANCOMYCIN – RESISTANT ENTEROCOCCUS ISOLATED FROM SURFACE AND GROUND WATER SAMPLES

D.P. Matlou[1], *A. Collins Njie*[2]
[1]*NORTH WEST UNIVERSITY, Department of Biological Sciences, MAFIKENG, South Africa*
[2]*North-West University- Mafikeng Campus, Department of Biological Sciences, Mmabatho, South Africa*

Backgrounds

Vancomycin Resistant *Enterococcus* (VRE) has been responsible for numerous outbreaks of serious infections in humans worldwide. *E. faecium* and *E. faecalis* are usually associated with Vancomycin resistance determinants thus cause hospital and community acquired infections in humans.

Objectives

The study was aimed at determining the antibiotic resistance and virulence profiles of VREs isolated from water samples.

Methods

Methods: A total of 170 water samples were collected and analysed. Eighty one potential isolates were screened for characteristics of *Enterococcus* species using preliminary biochemical tests, PCR assays and sequence analysis. The antimicrobial resistance profiles of isolates against 10 antibiotics were determined and a dendrogram was generated to access the relatedness of the isolates. Isolates were screened for the presence of antibiotic resistance and virulence genes by Multiplex PCR.

Results: A total of 56 isolates were confirmed as *Enterococcus* species. Large proportions (78.6%) of the isolates were resistant to Vancomycin. Forty four VREs isolates were detected phenotypically and 16% and 3.6% of the isolates possessed the *vanA* and *vanB* genes respectively. Large proportions of the isolates were resistant to multiple antibiotics and isolates with MAR phenotype VAN-NAL-STR-CHIL-AMP-OXYTET-GEN-NIT-SMX were resistant to eight antibiotics. Cluster analysis revealed two major clusters (Cluster 1 and Cluster 2). Sixteen (36.4%), 14 (27.3%), 3 (6.8%) and (4.5%) of the isolates possessed the *gel*, *asa*1, *hyl* and the *esp* virulence genes. None of the isolates possessed the *cylA* gene.**Conclusions**

Multiple antibiotic resistant enterococci that were also resistant to vancomycin and possessed virulence factors thus highlight the need for continuous monitoring.

FEMS7-2627
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL MONITORING FOR THE IMPACT OF COMBINED SEWER OVERFLOW INTO URBAN WATERWAY IN JAPAN, AND ITS REMEDIAL EFFECTS BY BUILDING A STORMWATER STORAGE TUNNEL.

K. Matsui[1]
[1]*Kindai University, Department of Civil and Environmental Engineering, Higashiosaka, Japan*

Backgrounds

A combined sewer system collects domestic sewage and rainwater into one pipe. However, the volume of wastewater exceeds the capacity of sewer pipe during heavy rainfall events. When this occurs, untreated sewage discharges directly into rivers as combined sewer overflow (CSO).

Objectives

To evaluate the effectiveness of newly commissioned stormwater storage tunnel, we explore the impact of CSO on the water quality of an urban waterway running through thickly populated Osaka-city, Japan.

Methods

We characterized the bacterial community of the Higashiyokobori-gawa waterway using next-generation DNA sequencing (Illumina Miseq). We examined bacterial 16S rRNA gene amplicons during rainfall periods.

Conclusions

When rainfall exceeded 4 mm/h at our experimental site on the waterway, the bacterial populations in the river water exhibited transient changes attributable to the CSO. The bacterial populations in the waterway differed greatly from those in sewage, suggesting that the CSO had been diluted with bulk rainwater prior to discharge into the river. After a newly constructed stormwater storage tunnel (i.e. underground storage capacity) was commissioned, we found no changes in the bacterial community in river water even when rainfall exceeded 8 mm/h. This reveals that the CSO was a key factor for the bacterial community changes. The result also indicates that the storage tunnel effectively prevented CSO discharge into the waterway. We suggest that bacterial community composition analysis is useful when monitoring water quality.

FEMS7-1409
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

VIBRIO CHOLERAE HMGA-MEDIATED PYOMELANIZATION CONFERS RESISTANCE TO PREDATION BY ACANTHAMOEBA CASTELLANII

D. McDougald[1], P. Noorian[2], Z. Chen[3], S. Kjelleberg[4], M. Wilkins[3], S. Sun[1]
[1]University of Technology Sydney, ithree Institute, Sydney, Australia
[2]University of New South Wales, School of Biotechnology and Bimolecular Science, Sydney, Australia
[3]University of New South Wales,
Systems Biology Initiative- School of Biotechnology and Biomolecular Sciences, Sydney, Australia
[4]Nanyang Technological University, Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore

Backgrounds

Predation by heterotrophic protists is one of the main biological factors constraining bacterial growth in aquatic environments, and thus has led to the evolution of a number of defence mechanisms that protect the bacteria from predation. These mechanisms may also function as virulence factors in infection of animal and human hosts, thus leading to a pathogenic lifestyle

Objectives

In order to identify the genes expressed by *V. cholerae* during predation by *Acanthamoeba castellanii,* whole transcriptome shotgun sequencing was performed.

Methods

Analysis of the transcriptome of grazed *V. cholerae* biofilms revealed that 197 transcripts were differentially regulated when compared to the non-grazed control. Differentially regulated transcripts included transcripts involved in biosynthetic and metabolic pathways, flagellar assembly and biosynthesis, iron transport, outer membrane proteins, and transcriptional and translational regulators.

Conclusions

The transcripts of genes involved in tyrosine metabolism were down-regulated in the grazed population, which indicates that the tyrosine metabolic regulon (VC1344-1347) may have a role in the response of *V. cholerae* biofilms to *A. castellanii* predation. Indeed, a pigmented mutant, disrupted in VC1345 (*hmgA*), was more grazing resistant to amoebae than the wild type. The increased grazing resistance was due to increased production of pyomelanin and thus reactive oxygen species.

FEMS7-2786
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL AND PHYSICAL-CHEMICAL CHARACTERIZATION OF NATURAL WATER RESOURCES FROM COASTAL AND ANDEAN REGIONS IN ECUADOR

D. Vinueza[1], J. Carrera[1], L. Mejia Castaneda[1], V. Ochoa Herrera[2], A.G. Pazmiño[2], A. Machado[1]
[1]Universidad San Francisco de Quito, Institute of Microbiology, Cumbayá, Ecuador
[2]Universidad San Francisco de Quito, Colegio de Ciencias e Ingeniería, Cumbayá, Ecuador

Backgrounds

One of the major worldwide health problems is the contamination of drinking water sources with toxic compounds and pathogenic bacteria. Some pathotypes of *Escherichia coli*, *Campylobacter*, *Legionella*, *Shigella* and *Salmonella* spp are causing global serious health problems.

Objectives

This study aims to analyze the quality of natural water resources in urban areas in Ecuador based on microbial and physical-chemical parameters, in order to compare the Costal and Andean regions and evaluate possible correlations between these parameters.

Methods

Escherichia coli and total coliforms quantification was conducted through growth media and PCR analysis for each of the aforementioned genera and *E. coli* pathotypes in triplicate samples from six different rivers. Meanwhile, environmental parameters in surface waters such as pH, conductivity, dissolved oxygen, chemical oxygen demand (COD), and total suspended solids (TSS) were determined following standard protocols.

Conclusions

Escherichia coli and total Coliforms counts showed the highest indices in samples from Guayllabamba, Machangara and Zamora rivers (Andean Region). Although samples from the Costal Region (Guayas, Chone and Esmeraldas rivers) showed lower numbers of *E. coli* and total Coliforms, both regions evidenced the presence of the same *E. coli* pathotypes (EIEC, EPEC and EAEC). On the other hand, the presence of *Shigella*, *Salmonella* and *Campylobacter* spp. was not detected, while *Legionella*was found in all samples. In the case of Guayas and Zamora rivers, TSS and COD values exceeded the maximum permissible limits from Ecuadorian legislation. Finally, a positive correlation was found between the COD concentration and the presence of *E. coli* and Coliforms.

FEMS7-2038
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

AN EVOLUTIONARY APPROACH TO DETECT THE MAXIMUM POTENTIAL ADAPTATION OF MICROCYSTIS AERUGINOSA TO SALINITY

I.J. Melero Jiménez[1], E. Martín-Clemente[1], M.J. García-Sánchez[1], A. Flores-Moya[1], E. Bañares-España[1]
[1]Facultad de Ciencias- Universidad de Málaga, Departamento de Biología Vegetal, Málaga, Spain

Backgrounds

Salinity is increasing in many inland waters, on a world-wide scale, due to agricultural practices, droughts, or rise in sea level. In order to understand the effects of increased salinity on freshwater ecosystems, the cosmopolitan toxic cyanobacterium *Microcystis aeruginosa* (Kützing) Kützing was selected as a model. It is known that this species that this species cannot proliferate in salinity >10-15 $g \cdot L^{-1}$ but the limit of the adaptation to salinity remains to be investigated.

Objectives

To detect the maximum genetic adaptation capacity of three strains of *M. aeruginosa* (isolated from a freshwater reservoir with salinity <0.025 $g \cdot L^{-1}$, i.e. without previous "evolution salinity history") to the exposure of increased salinity.

Methods

An experimental evolutionary approach (ratchet experiment) was used. In order to analyze the differential evolutionary potential of three *M. aeruginosa* strains, two ratchet experiments were performed based on "soft" or "intense" exponentially increased salinity.

Conclusions

Acclimation (supported by the gene expression already present in the ancestral populations) was similar in the three strains in both ratchets experiments ("soft" and "intense"). A significant enhancement in resistance, supported by the selection of new genetic variants arising by mutations (genetic adaptation), was achieved in all derived populations in both ratchet experiments, and in the three strains. However, the results showed that the dynamics and the limit of genetic adaptation depend on previous adaptation history (historical contingency).

FEMS7-2188
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TEMPORAL VARIABILITY OF OSCILLATORIA SP. IN EXTREME ENVIRONMENT: A SULFIDE-RICH SPRING OUTFLOW

A. Reul[1], E. Martín-Clemente[2], I.J. Melero Jiménez[2], M. Hernández-López[3], F.J. Toro[2], E. Bañares-España[2], A. Flores-Moya[2], M.J. García-Sánchez[2]
[1]Facultad de Ciencias- Universidad de Málaga, Departamento de Ecología y Geología, Málaga, Spain
[2]Facultad de Ciencias- Universidad de Málaga, Departamento de Biología Vegetal, Málaga, Spain
[3]Facultad de Ciencias- Universidad de Málaga, Departamento de Química analítica, Málaga, Spain

Backgrounds

The Hedionda Spa (Andalucía, southern Spain) is a sulfide-rich spring outflow, but despite the inhibitory effect of sulfide on photosynthesis, a phytoplankton community inhabits this extreme environment.

Objectives

To analize the phytoplanktonic groups present in La Hedionda Spa along an annual cycle, and to study the possible correlation between sulphide concentration and the presence of different groups.

Methods

A monthly and weekly sampling was started on March 2016. Samples were analyzed with a fluorospectrometer to detect the presence of different groups. A FlowCAM analysis of photoplankton was carried out at 5-100 µm ESD to determine biovolume and abundance. Water samples were analized to determine sulphide concentration.

Conclusions

Sulphide concentrations varied from 0.8 to 219.4 µM, while chlorophyll *a* ranged between 0.4 and 11.7 ug l^{-1}. Cyanobacteria is the predominant phytoplanktonic group, accounting for 50-90% of total chlorophyll. *Oscillatoria* sp. is the predominant cyanobacteria in terms of abundance (0.55-65.6 filamemts·ml^{-1}) and biovolume ($9 \cdot 10^5$-$3 \cdot 10^8$ μm^3 ml^{-1}). A positive correlation was found between sulphide concentration and *Oscillatoria* sp. abundance, but not between sulphide and total chlorophyll *a* concentration. The positive correlation could be due to (i) dilution of both sulphide and *Oscillatoria* sp. abundance by runoff, or (ii) to a physiological advantage of *Oscillatoria* sp. under elevate sulphide, which has been confirmed in laboratory experiments. However, high sulphide might also reduce *Oscillatoria* sp. growth rate, but the absence of potential predator or competitor provides *Oscilatoria* sp. an advantage in the environment not detectable in laboratory approaches.

FEMS7-2505
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CULTURABLE DIVERSITY OF HALOPHILIC ARCHAEA ISOLATED FROM HYPERSALINE ENVIRONMENTS IN NORTHEASTERN ALGERIA

T. Menasria[1,2], L. Benammar[2], A. Ayachi[2], H. Hacène[3], M. Monteoliva-Sánchez[1], M. Aguiler[1]
[1]Faculty of Pharmacy, Department of Microbiology- University of Granada s/n 18071-, Granada, Spain
[2]Faculty of Natural and Life Sciences,
Department of Microbiology and Biochemistry- University of Batna 2- 05078, Batna, Algeria
[3]Faculty of Biological Sciences,
Departement of Cellular and Molecular Biology- University of Sciences and Technology Houari Boum ediene USTHB, Algiers, Algeria

Backgrounds

Algeria includes a multitude of wetlands with rare typology and ecology in the world, where 50 sites are classified as Ramsar sites of international importance. However, very original, diverse and typical extreme environments including saline lakes, salterns, saline and hypersaline soils in the steppes and Sahara, which owns 80% of the country land area, still be little studied for assessing their biological resources especially in term microbial diversities.

Objectives

Our research work deals with the issue of halophilic microbial diversity in a representative arid and desertic areas (hot hyper-lands) of South and Eastern Algeria. Thus, to establish a model of knowledge of the halophilic Archaeal communities in salt lakes (Chott and Sebkha)

Methods

In this study, the diversity of halophilic archaea from different chotts and sebkha (dry salt lakes and salt flat) in northeastern Algeria, was investigated using media supplemented with different salt concentration (15, 20, 25 and 30%) based on recognized halophilic techniques followed by preliminary identification analyzes and determination of some biological activities (hydrolytic enzyme, antibacterial activities). More than 70 halophilic archaea were isolated and preliminary polyphasic study with different conventional phenotypic tests (physiological, morphological and cultural) associated with 16S rRNA genes and phylogenetic analysis were done for the typing of the isolates.

Conclusions

Thus, the biodiversity found was considered wide in these environments, and sequencing of the 16S rRNA genes and phylogenetic analysis allowed the identification of possible novel species from different Archaeal genera such as Haloferax, Haloarcula, Halogeometricum, Natrinema and Natrialba,

FEMS7-2885
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

PHYLOGENETIC DIVERSITY OF RARE HALOPHILIC ACTINOMYCETES ISOLATED FROM SALINE SOILS IN NORTH-EASTERN ALGERIA

T. Menasria[1,2], L. Benammar[2], A. Ayachi[2], H. Hacène[3], M. Monteoliva-Sánchez[1], M. Aguilera[1]
[1]Faculty of Pharmacy- University of Granada, Department of Microbiology, Granada, Spain
[2]Faculty of Natural and Life Sciences- University of Batna 2,
Department of Microbiology and Biochemistry, Batna, Algeria
[3]University of Sciences and Technology HouariBoumediene,
Departement of Cellular and Molecular Biology, Algiers, Algeria

Backgrounds

Hypersaline environments present extreme conditions for life, however they are present in many regions throughout the world. In Algeria, there are several hypersaline sites with rare and unique typology without characterization or very scarce microbial investigation. Therefore, the archaeal and bacterial diversity including actinomycetes of these extreme saline environment remains poorly understood. Moreover, due to the antibiotic resistance global health problem, a renewed interest in the searching and development of new products with interesting biological activity is being actively pursued.

Objectives

To identify the Actinomycetes microbiota present in these extreme and unexplored environments. They could be targeted as new sources of structurally diverse natural products with new significant biological activity and potential biopharmaceutical applications

Methods

In this study, isolation of halophilic actinomycetes from different chotts and sebkha in northeastern Algeria, was investigated using media supplemented with different salt concentration based on recognized halophilic culture techniques followed by preliminary identification and determination of somebiological activities (hydrolytic enzymes, antibacterial activities). A total of 23 halophilic actinonmycetes isolates were recovered from different selected Algerian soils sampling. Sequencing and analysis of 16S rRNAgenes from representative isolates displayed the presence of members affiliated to rare actinobacterial genera: *Nocardiopsis and Actinonpolyspora.*

Conclusions

To the best of our knowledge, this study constitutes the first characterization of rare actinomycete diversity on salterns and hypersaline soils located in the eastern region of Algeria. Appropriate identification of rare actinomycetes species retrieved from the Algerian saltern soil samples will contribute to laid a platform to search for novel biotechnologically significant bioactive substances.

FEMS7-2138
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DEVELOPMENT OF A CONSORTIUM OF BACTERIAL STRAINS ISOLATED FROM A SANDY BEACH WITH THE ABILITY TO DEGRADE PETROLEUM HYDROCARBONS

R. P. Mendes[1,2,3], *F. Santos*[1], *C.M. R.Almeida*[1], *M.F. Carvalho*[1], *A.P. Mucha*[1]
[1]*Interdisciplinary Centre of Marine and Environmental Research, EcoBiotec, Matosinhos, Portugal*
[2]*ICBAS/UP - Institute for the Biomedical Sciences Abel Salazar/ University of Porto, ICBAS, Porto, Portugal*
[3]*Faculty of Sciences of University of Porto, Porto, Portugal*

Backgrounds

Oil spills from anthropogenic sources pose a serious threat to coastal ecosystems, requiring prompt mitigation measures. There is thus a need for the development of cleaner and more efficient remediation techniques. Bioremediation has proven to be an eco-friendly and promising remediation technique, especially when using autochthonous microorganisms.

Objectives

In this line, we aimed to develop consortia of autochthonous bacterial strains, capable of degrading petroleum hydrocarbons, which could be used for the bioremediation of oil spills.

Methods

Thus, two different consortia were prepared, each constituted by a mixture of 5 bacterial strains previously isolated from a sandy beach (Cabo do Mundo) in the NW Portuguese coast, cultivated in Bushnell – Haas broth (BH) in the presence of two different sources of carbon (crude oil or sodium acetate).

Then, microcosm experiments were performed in flasks containing beach sediment, BH and crude oil, for 15 days, under three different conditions: natural attenuation, biostimulation (through the addition of nutrients) and bioaugmentation (through the addition of the previous consortia) combined with biostimulation. Samples were taken at the beginning and end of the experiment to assess hydrocarbon degrading microorganisms through an adapted most probable number method. Analysis of total hydrocarbons by FT/IR, to evaluate their degradation along time, was also carried out.

Conclusions

This study provides an insight of the natural community capacity to degrade hydrocarbons and its possible future application in bioremediation techniques. In this way, it is possible to act locally and ecologically facing an eventual oil spill incident.

Acknowledgments - project NORTE-01-0145-FEDER-000035 (ERDF) and ICBAS (UP).

FEMS7-2442
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

OVEREXPRESSION OF ISIB FLAVODOXIN IN BURKHOLDERIA XENOVORANS CONTRIBUTES TO ENHANCED TOLERANCE TO HYDROGEN PEROXIDE AND AROMATIC DEGRADATION

V. Mendez[1], *A. Moyano*[2], *A. Smania*[2], *M. Seeger*[1]
[1]*Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química & Centro de Biotecnología, Universidad Técnica Federico Santa María, Valparaíso, Chile*
[2]*Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina*

Backgrounds

Long-chain flavodoxins (Flds), electron carrier flavoproteins that contain a flavin mononucleotide as prosthetic group, play a key protective role against reactive oxygen species (ROS) in diverse microorganisms. Flds, largely isofunctional with the electron carrier ferredoxins, are sensitive to ROS and its overexpression has been shown to confer increased tolerance to stress in plants and bacteria. However, Flds are restricted to specific prokaryotes and oceanic algae.

Objectives

To study the effects of flavodoxin IsiB from *Anabaena variabilis* overexpression in the model aromatic compounds-degrading strain *Burkholderia xenovorans* LB400 under oxidative stress conditions

Methods

In this study, we overexpressed flavodoxin IsiB from the cyanobacterium *Anabaena variabilis* in *Burkholderia xenovorans* LB400, a model aromatic compounds-degrader bacterium. We analyzed the effects of flavodoxin IsiB in bacterial survival and ROS formation upon hydrogen peroxide exposure. Growth on aromatic compounds by recombinant cells was also studied, a condition that leads to oxidative stress.

Conclusions

Cyanobacterial IsiB conferred increased tolerance to hydrogen peroxide and decreased ROS formation upon exposure for 1 h (10 and 20 mM). Moreover, IsiB contributed to bacterial survival after 10 mM hydrogen peroxide exposure for 1 h. Growth of recombinant *B. xenovorans* cells on aromatic compounds was also tested. Interestingly, turbidity of IsiB-overexpressing cells was higher when using 3- and 4-hydroxyphenylacetate as sole carbon sources in minimal media. Flavodoxin IsiB contributed in an enhanced response to oxidative stress and degradation of aromatic compounds in *B. xenovorans*.

Acknowledegments: PhD CONICYT fellowships (VM), FONDECYT (1151174 & 1110992), RIABIN and USM (131562 & 131342) grants.

FEMS7-2397
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SPATIO-TEMPORAL DYNAMICS OF THE MICROBIAL COMMUNITIES IN GYPSUM-RICH EXTREME ENVIRONMENTS (MONEGROS DESERT, SPAIN)

M. Menendez Serra[1], X. Triadó-Margarit[1], C. Castañeda[2], J. Herrero-Isern[3], E. O Casamayor[1]
[1]Centre for Advanced Studies of Blanes CEAB- Spanish Research Council CSIC, Ecología Continental, Blanes, Spain
[2]Estación Experimental de Aula Dei- Spanish Research Council CSIC, Soil Management and Global Change, Zaragoza, Spain
[3]Estación Experimental de Aula Dei- Spanish Research Council CSIC, Irrigation- Agronomy and Environment, Zaragoza, Spain

Backgrounds

The Monegros desert contains one of the largest sets of aquatic and soil saline environments in Europe forming a unique landscape of great scientific and ecological value. The uniqueness of these saline and gypsum-rich environments has been documented in geologic, hydrologic, mineralogic and edaphic studies providing habitats for rare and threatened plants and animals. A large number of endemisms have been reported mainly of invertebrates, vascular plants, lichens and bryophites. The genetic identity, novelty and temporal dynamics of the microbial component remains poorly studied.

Objectives

To describe the composition and distribution of microbial communities in saline environments of the Monegros desert and Gallocanta lagoon and the underlying patterns behind microbial community colonization, assembly and diversity.

Methods

The Monegros Desert is located within the semi-arid Central Ebro Basin (41°42′N, 0°20′W), and includes an endorheic area (c. 400 km2) with 149 scattered saline depressions. The basins are excavated on gypsum-rich bedrock and range in size from < 2 to 239 ha. Gallocanta is a large endorheic rain-fed shallow lagoon (14.4 km2, 50 cm deep) located in a plateau 1000 m a.s.l. in the Iberian Mountain system (40°58′N 1°29′W). DNA was extracted from soil and water samples along a temporal survey, and PCR amplicons were analysed by high-throughput sequencing Illumina and cloning and sequencing techniques.

Conclusions

The microbial genetic diversity was broad and with remarkable novelty with recurrent presence of bacteria initially isolated from polar marine habitats, and new groups of Archaea. Microbial eukaryotes were substantially different from protists and fungi previously reported from marine or freshwater. The great scientific and ecological value found for macroorganisms can be extended to the idiosyncratic microorganisms inhabiting such unique habitat in Europe.

FEMS7-1878
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

INTER-LABORATORY CALIBRATION OF QUANTITATIVE ANALYSES OF ANTIBIOTIC RESISTANCE GENES

J. Rocha[1], D. Cacace[2], H. Guilloteau[3], T. Jäger[4], R. Marano[5], C.M. Manaia[1], C. Merlin[3], D. Fatta-Kassinos[6], E. Cytryn[5], J. Slobodnik[7], T.U. Berendonk[2], T. Schwartz[4], V. Dulio[8]
[1]CBQF - Centro de Biotecnologia e Química Fina,
Universidade Católica Portuguesa- Escola Superior de Biotecnologia, Porto, Portugal
[2]Environmental Sciences Technische Universität Dresden, Institute for Hydrobiology, Dresden, Germany
[3]Laboratory of Physical Chemistry and Microbiology for the Environment, Nancy University, Nancy, France
[4]Karlsruhe Institute of Technology, Institute of Functional Interfaces, Karlsruhe, Germany
[5]Agricultural Research Organization,
Institute of Soil Water and Environmental Sciences Volcani Center, Bet Dagan, Israel
[6]Nireas-
International Water Research Centre and Department of Civil Engineering and Environmental Engine ering, University of Cyprus, Nicosia, Cyprus
[7]Environmental Institute, Environmental Institute, Kos, Slovak Republic
[8]INERIS - Direction Risques Chroniques Rue Jacques Taffanel Parc technologique ALATA,
Executive Secretary of the NORMAN Association, Verneuil-en-Halatte, France

Backgrounds

Antibiotic resistant bacteria and antibiotic resistance genes (ARGs) are major human-health threats, widely distributed in the environment. Quantitative PCR (qPCR) is a standard approach to detect and quantify ARGs in environmental compartments. However, the comparison of gene quantification reported by different laboratories is challenging since data are predominantly obtained under non-harmonized conditions, using different qPCR protocols.

Objectives

The aim of this study was to develop and calibrate standardized qPCR procedures for quantification of key ARGs, analyzing the same samples with common protocols and distinct equipment, reagents batches and operators.

Methods

Treated wastewater from three European countries were processed immediately after collection and transported to the laboratory for total DNA extraction. DNA extracts from each sample were pooled and aliquots were distributed by five partners involved in the calibration procedure. The genes 16S rRNA, *vanA*, bla_{TEM}, *qnrS*, *sul1*, $bla_{CTXM-32}$ and *intI*1 were analyzed using harmonized qPCR protocols and the constructed pNORM1 plasmid, which contains fragments of the seven targeted genes, was used for generating standard curves.

Conclusions

The 16S rRNA gene was the most abundant, followed by *sul1*, *intI*1, *qnrS* and bla_{TEM}. Quantifications made by different partners were reproducible and inter-laboratory variation was < 20%. The notorious exception was for the *qnrS* gene, and therefore protocol improvement is recommended. The genes $bla_{CTXM-32}$ and *vanA* were below the limit of quantification in most or all of the samples analyzed. The inter-laboratory calibration is an adequate approach to reliably assess ARG abundance and environmental contamination in different environments and geographic locations.

FEMS7-1165
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

FROM FIELD TO FIELD: USING ENDOPHYTIC AND RHIZOSPHERIC BACTERIA TO ENHANCE ARSENIC PHYTOREMEDIATION BY AUTOCHTHONOUS BETULA CELTIBERICA

V. Mesa[1], A. Navazas[2], R. González-Gil[2], A. González[2], N. Weyens[3], B. Lauga[4], J.L. R. Gallego[5], J. Sánchez[1], A.I. Peláez[1]
[1]University of Oviedo, Department of Functional Biology, Oviedo, Spain
[2]University of Oviedo, Department of Organisms and Systems Biology, Oviedo, Spain
[3]Hasselt University, Centre for Environmental Sciences CMK, Hasselt, Belgium
[4]Université de Pau et des Pays de l'Adour, Equipe Environnement et Microbiologie EEM, Pau, France
[5]University of Oviedo, Department of Exploitation and Mining Prospecting, Mieres, Spain

Backgrounds

Asturias (NW Spain), is characterized by the existence of a significant number of degraded areas from the dismantling of chemical industry. These anthropogenic activities have generated waste with high metal(loid)s content, among them arsenic is one of the most hazardous element. A representative example of a large-scale contaminated site is *Nitrastur*, which is a highly-contaminated abandoned fertilizer industry. *Betula celtiberica* is a deciduous tree, pseudometallophyte, and fast-growing high biomass plant colonizing the study area.

Objectives

The potential of indigenous arsenic-tolerant bacteria to enhance arsenic-phytoremediation using as accumulator plant the autochthonous *Betula celtiberica* was addressed.

Methods

Analysis of the whole rhizosphere and endophytic bacterial communities, the cultivable species and subsequent bioaugmentation experiments.

Conclusions

Betula celtiberica's microbiome was dominated by taxa related to *Flavobacterium, Burkholderiales* and *Pseudomonas*. A total of 54 cultivable rhizobacteria and 41 root endophytes, mainly affiliated to the phyla *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Actinobacteria* were isolated and characterized with ability to promote plant growth and to mitigate heavy metal stress. Seven bacterial isolates were selected and tested for *in vitro* arsenic plant-accumulation and four of them were finally tested in a field-scale study. The exposure to arsenic *in vitro* caused an increase in content of total non-protein thiol compounds in roots, suggesting a detoxification mechanism through phytochelatins complexation. In the field, an endophytic bacterial consortium enhanced As-accumulation in leaves and roots, whereas the rhizosphere isolate *Ensifer adhaerens* strain 91R mainly promoted plant growth. Field experimentation showed that additional factors, arsenic content and pH of soil, influenced the plant arsenic uptake.

FEMS7-1278
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

BACTERIAL, ARCHAEAL, AND EUKARYOTIC DIVERSITY ACROSS ECOLOGICAL NICHES IN LOS RUELDOS ACID MINE DRAINAGE

V. Mesa[1], C. Méndez-García[2], R. González-Gil[3], J.L. R. Gallego[4], J. Sánchez[1], A.I. Peláez[1]
[1]University of Oviedo, Department of Functional Biology, Oviedo, Spain
[2]University of Illinois, Carl R. Woese Institute for Genomic Biology, Urbana, USA
[3]University of Oviedo, Department of Organisms and Systems Biology, Oviedo, Spain
[4]University of Oviedo, Department of Exploitation and Mining Prospecting, Oviedo, Spain

Backgrounds

Acid mine drainages are characterized by their low pH and the dissolved toxic metallic species. Los Rueldos, an abandoned mercury mine has originated an environment typical of acid drainage, where a very specific set of geochemical conditions act as key in the development of a unique microbial community.

Objectives

We surveyed the microbial diversity in all domains of life in different niches (acid water, sediments, and biofilms), and predicted bacterial function based on community composition.

Methods

The analysis was addressed through phylogenetic analysis based on 18S rRNA gene and large-scale parallel pyrosequencing of 16S and 18S.

Conclusions

Sediment samples contained higher proportions of soil bacteria (AD3, Acidobacteria), Crenarchaeota, and Methanomassiliicoccaceae archaea. Subaerial biofilm at the interface rock/acid water and biofilm at the interface sediment/water samples, were enriched in iron oxidizers (genus *Leptospirillum*), Acidithiobacillales, Betaproteobacteria, and Thermoplasmata archaea. Water samples were enriched in Cyanobacteria and Thermoplasmata archaea at 3-98% of the sunlight influence, while Betaproteobacteria, Thermoplasmata, and Micrarchaea dominated in acid water collected in total darkness. Stalactites hanging from the Fe-rich ceiling samples were dominated by the neutrophilic iron oxidizer *Gallionella* and lineages absent in the rest of micro-habitats (Chlorobi, Chloroflexi). Eukaryotes were detected in biofilms and outdoors water samples, and belonged mainly to clades SAR (Alveolata and Stramenopiles), and Opisthokonta (Fungi). Acidic biofilm with uppermost oxic and hypoxic biofilms displayed higher proportions of ciliates (Gonostomum, Oxytricha), whereas outside water samples were enriched in fungi (*Paramicrosporidium* and microbial Helotiales). Predicted function through bacterial community composition suggested adaptive evolutive convergence of function in heterogeneous communities.

FEMS7-1429
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

AGROBACTERIUM FABRUM HYDROXYCINNAMIC ACIDS DEGRADATION REGULATION: OPTIMIZATION FOR CONTRASTING LIFESTYLES?

T. MEYER[1], S. RENOUD[1], I. KERZAON[1], A. VIGOUROUX[2], V. GAILLARD[1], S. MORERA[2], L. VIAL[1], C. LAVIRE[1]
[1]UMR Ecologie Microbienne- CNRS- INRA- VetAgroSup- UCBL- Université de Lyon, Biology, Villeurbanne, France
[2]Institute for Integrative Biology of the Cell I2BC- CNRS CEA University Paris-Sud, Biophysics- Biochemistry and Structural Biology, Gif-sur-Yvette, France

Backgrounds

Hydroxycinnamic acids (HCA) are plant compounds, precursor of lignin synthesis and moreover toxic for certain bacteria. However, in *Agrobacterium fabrum* strains, HCA are degraded and used as carbon sources thanks to a species specific genes cluster, absent from the other *Agrobacterium* species. This degradation pathway and its genomic organization are original and contains both the genes involved in the degradation process and their putative regulator (*hcaR*), a MarR family regulator (Campillo et al., 2014, Lassalle et al., 2011). Regulation allows to optimize the cell response and to narrow the fitness expression cost in changing environments. *A. fabrum* HCA degradation species specific genes have been proposed to be selected by natural selection for their importance in plant-bacteria interaction. However, *Agrobacterium* has two different lifestyles both in interaction with plant: either commensal living in plant rhizosphere, or pathogenic by creating its own ecological niche after plant cells transformation leading to tumor formation (crown gall disease).

Objectives

The aim is to investigate the molecular and ecological role of *A. fabrum* HcaR in the two contrasting lifestyles of the bacteria.

Methods

We studied the HcaR regulation by molecular approaches (deletion mutant, transcriptional fusion, gel-shift assays). The implication of HcaR in bacterial fitness *in planta* was studied by gene expression and competition assays.

Conclusions

We described the HcaR regulation of HCA degradation genes and demonstrated that it is an important phenomenon for *A. fabrum* interaction with plants. Contrasting results of the fitness cost associated to HcaR deletion were observed for the two lifestyles of *Agrobacterium*.

FEMS7-1019
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ECOTOXICOLOGICAL EVALUATION AND MACROLIDE-RESISTANCE DIVERSITY OF CROATIAN PHARMACEUTICAL EFFLUENTS

M. Milaković[1], A. Šimatović[2], J.J. Gonzalez Plaza[1], A. Bielen[3], I. Senta[1], T. Jurina[4], S. Babić[5], N. Udiković Kolić[1]
[1]Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia
[2]Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia
[3]Faculty of Food Technology and Biotechnology, Department of Biochemical Engineering, Zagreb, Croatia
[4]Faculty of Food Technology and Biotechnology, Department of Process Engineering, Zagreb, Croatia
[5]Ruđer Bošković Institute, Division of Materials Chemistry, Zagreb, Croatia

Backgrounds

Effluents from antibiotic manufacturing are recognised as the most important contributors to aquatic pollution with antibiotics which could have negative impact on biological balance of natural environments and could also facilitate the development and dissemination of antibiotic resistance.

Objectives

The objective of this study was to characterize effluents from a local pharmaceutical industry manufacturing the macrolide antibiotic azithromycin with respect to its antibiotic content, ecotoxicity and diversity of macrolide-resistance genes.

Methods

The effluents were collected on two occasions in 2016, in winter and in spring. LC-MS analysis of effluents showed high concentrations of azithromycin, N-desmethyl azithromycin and dehydrated erythromycin (in the range of mg/L). Ecotoxicity tests showed high toxicity of effluents to diverse freshwater organisms (algae, *Daphnia* and zebrafish embryos). Culturing showed that effluents contained high frequency of azithromycin-resistant bacteria (> 70%). Functional metagenomics identified two main macrolide resistance mechanisms which include antibiotic efflux and inactivation. Some macrolide resistance genes are flanked by mobile genetic elements such as IS elements suggesting that they are candidates for dissemination to other bacteria in the environment, including pathogens.

Conclusions

Using a wide array of analyses we have demonstrated that discharges of local pharmaceutical industry may pose a significant ecological and public health concern due to their high toxicity to aquatic biota and the potential to promote global spread of antibiotic resistance.

FEMS7-0759
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

USE OF SUBSTRATES AND ENZYMATIC ACTIVITIES BY THE AQUATIC MICROBIAL ASSEMBLAGES OF ENDORHEIC LAKES ALONG A SALINITY GRADIENT

J. Miralles[1], A. Camacho[1], A. Picazo[1], C. Rochera[1], A. Camacho[1]
[1]Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Limnologia, Paterna, Spain

Backgrounds

Salinity is a paramount environmental variable shaping the microbial communities thriving in aquatic environments and their phenotypic characteristics.

Objectives

In this work, we aim to assess the community-level physiological profile (CLPP) and the enzymatic activities of the aquatic microbial community of 17 endorheic lakes located in La Mancha Húmeda Biosphere Reserve (Central Spain), showing an increasing salinity gradient among them and also through time.

Methods

The assessment was performed by using the Biolog EcoPlate system, which is especially designed for the analysis of the CLPP in soils and waters. We additionally measured two enzymatic activities (phosphatase and celobiase).

Conclusions

As a general pattern, salinity increases resulted in lower consumption but also in the use of a narrow variety of substrates. Our results also show a pattern of specialization on the CLPP with increasing salinity. Lakes with low salinity show a very similar pattern of substrate use, but with low consumption of each substrate. Contrarily the most saline lakes show a high consumption of just a few substrates, and this specialization grows towards the warmest periods, as do enzymatic activities.

FEMS7-1482
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ISOLATION AND CHARACTERIZATION OF CYANOTROPHIC AND ARSENIC OXIDIZING BACTERIUM PSEUDOMONAS SP. P6115

C. Hernández Rodríguez[1], *A. Miranda Carrazco*[1], *L. Villa Tanaca*[1]
[1]*Instituto Politécnico Nacional, Microbiología, Mexico City, Mexico*

Backgrounds

Mine wastes are man-made environments with high concentrations of heavy metals and cyanide but low concentration of organic matter, and inorganic and organic sources of combined nitrogen. Some pioneer bacteria can colonize and grow in mine wastes with several selection pressures and nutritional limitations.

Objectives

Identify and characterize of a cyanotrophic bacterium able to degrade cyanide and oxidize arsenic from mine wastes as a potential candidate for biological treatment of cyanide mine tailings.

Methods

A bacterium was isolated from waste tailings with high concentration of cyanide and it was identified as *Pseudomonas* sp. P6115 by phylogenetic analysis of 16S rRNA, *gyrB, rpoB* and *rpoD* genes. Also, the strain was tested to cyanide degradation and arsenic oxidation by in vitro physiological and molecular assays.

Conclusions

The growth kinetics of the strain in media with cyanide as a sole nitrogen source was associated to cyanide removal. Also, ferricyanide, a component of wastewater, was used as a sole nitrogen source. The strains tolerated arsenite and arsenate, and exhibited capacity to oxidize arsenite. *Pseudomonas* sp. P6115 can be proposed as candidate for the biological treatment of cyanide and arsenic in mine wastes.

FEMS7-2794
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

A PILOT CORRELATION STUDY: HOW GENETIC VARIATION REGULATES WITHIN-POPULATION DIFFERENCES IN THE GUT MICROBIOTA OF GIANT PANDA?

S. Mishra[1], J. Tang[1], Y. Li[1]
[1]SICHUAN AGRICULTURAL UNIVERSITY,
Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, CHENGDU, China

Backgrounds

For an enigmatic species like the bamboo-eating giant panda, which still retains a carnivore-like short gastrointestinal tract, it becomes extremely significant to examine its gut microbiota diversity for its health status and future survival. Gut microbiota composition and diversity vary within and among populations and several factors have been reported to play important roles in shaping the gut microbiota at different taxonomic scales. Therefore, to understand variations in gut microbial communities, we must determine how processes regulating microbial community assembly (colonization, persistence) differ among hosts and affect microbiota composition.

Objectives

Hence, we examine the correlation of structural composition of gut microbiota with genetic diversity in wild giant pandas.

Methods

Eighty fecal samples of wild giant pandas were collected from Wolong National Nature Reserve, China. Further, we investigated the structural profile of the giant panda gut microbiome comprehensively based on pyrosequencing of V4-V5 region of 16S rDNA and assessed genetic diversity using primers for Cyt-b gene and D-loop region of mitochondrial DNA.

Conclusions

The bacterial composition in the wild giant pandas was primarily dominated by *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* (contributing 60.49%, 21.85% and 14.95%, respectively). We also found within-population differences in the gut microbiota of Wolong pandas. Furthermore, we attempted to correlate within-population differences in gut microbiota with genetic variation in pandas. Here we discuss the first attempt to identify and partition colonization and sorting process in a gut microbiota metapopulation of the wild giant panda with large sample-size. This study would help in developing effective future conservation plans.

FEMS7-3243
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

USING MICROBIAL SOURCE TRACKING MARKERS TO PREDICT OCCURRENCE OF WATERBORNE PATHOGENS IN URBAN AND AGRICULTURAL WATERSHED

M. Molina[1]
[1]*US Environmental Protection Agency, ORD, Athens, USA*

Backgrounds

Runoff from agricultural fields and urban landscapes may carry a variety of microbial contaminants that compromises water quality and increases the possibility of human exposure to pathogenic microorganisms.

Objectives

The objective of this study was to predict occurrence of waterborne pathogens using measurements of fecal indicator bacteria (FIB), microbial source tracking (MST) markers and environmental parameters to support development of risk assessment models and gain better understanding of pathogen behavior in mixed use watersheds.

Methods

Collection of stream water and sediment samples was conducted from November 2012 to December 2013 in an agricultural watershed in N.E. Georgia, USA and from November 2015 through December 2016 in an urban watershed (Atlanta, Georgia). *E. coli, Enterococcus sp., Salmonella (invA)*, *Shiga toxin* producing pathogens (*Stx2*), and MST markers were measured in over 25 locations across both watersheds. Decision tree analysis was used to determine independent variables describing the occurrence of waterborne pathogens.

Conclusions

Salmonella, and Stx2 were detected in 76.2%, and 61.1% of water samples and in 51.1%, and 43.3% of sediment samples, respectively. Water *E. coli* concentration, temperature, DO, pH and ruminant-associated marker concentration predicted presence of pathogens in agricultural watersheds. In the urban watershed, temperature, conductivity, and D.O predicted the presence of *Salmonella.* Culturable *E. coli* and the human associated marker were poor *Salmonella* predictors, suggesting that *Salmonella* may be originating from non-human sources. This study shows the utility of integrating microbial water quality measurements with MST markers to predict pathogen occurrences in streams impacted by mixed land uses.

FEMS7-2911
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

ANTARCTIC FUNGI DIVERSITY WITH BIOTECHNOLOGICAL APPLICATIONS

L. Monserrate-Maggi[1], M. Ratti-Torres[1], J. Vargas-Peréz[1], L. Serrano-Mena[1], I. Valderrama[1], J. Cevallos-Cevallos[1]
[1]Escuela Superior Politécnica del Litoral, Centro de Investigaciones Biotecnológicas del Ecuador, Guayaquil, Ecuador

Backgrounds

The study of microbial community was much advanced by recent introduction of Next Generation Sequencing (NGS) technologies, this has allowed to understand structures and relationships with biotic and abiotic factors of microbial community in extreme environments, in addition to the different uses in biotechnology.

Objectives

This study focused on the molecular identification and bioprospecting of cultured, and non-cultured fungal community from Antartic soils.

Methods

The samples were processed by molecular techniques including 454 pyrosequencing. Isolates were identified by PCR amplification and ITS1, 5.8S, and ITS2 region sequencing. Bioinformatic analyzes were performed using the Quantitative Insights Into Microbial Ecology software and compared to the UNITE version 7 database. Wealth and Shannon were calculated using pyrosequencing data. Yeast bioprospecting was performed by incubating each isolate at 20 ° C on mineral media enriched with urea, casein / gluten, starch, or fat to test for ureases, peptidases, amylases, or lipases respectively. Fermentation of sugars was tested using sucrose, fructose, glucose, maltose, lactose, and glycerol.

Conclusions

The presence of fungi in Antarctica is mainly due to their role in the primary decomposition of organic material, besides genres that increase in abundance at lower temperatures. This research combining fungal isolation with 454 pyrosequencing emphasizes the importance of the use of molecular methods of detection to obtain a more accurate description of diversity, although there are other sequencing technologies that are used today. The diversity of fungal genera in the Antarctic is still low relative knowledge. Finally, fermentation of *Candida* showing potential application in the production of beer.

FEMS7-3032
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIOLOGICAL VARIABLES AS SOIL QUALITY INDICATORS: EVALUATION OF AEROBIC TREATED MANURE APPLICATION IN AGRICULTURAL SOILS

A. Montanez[1], S. Vico[1], L. Nuñez[1], C. Silva[1], N. Rigamonti[1]
[1]Facultad de Ciencias, Montevideo, Montevideo, Uruguay

Backgrounds

Soil microbial properties related to the biodiversity and biocycles of soil nutrients, are indicators of soil quality, but there is still no consensus as to how they should be used.

Objectives

In this study we evaluated the impact of aerobic treated manure application on soil microbiological characteristics, with the aim of identify a minimal microbiological data set to monitor early changes in soil quality

Methods

The biofertilizer utilized in the experiment was obtained through *in situ* aerobic treatment of livestock manure. Two field sites were used, with high and low input application and soil samples were with and without application of biofertilizer. Soils pH, water content and rate of N mineralization were determined. Different microbial activities were measured: carbon soil microbial biomass; soil biodiversity, including the metabolic profile of soil microbial organism and number of cultivable mesophilic aerobic bacteria, fungi and actinomyces; soil enzyme activity; soil physical properties, related to glomalin concentration in soil; soil fertility, includes functional microbial groups with the ability to supply directly or indirectly essential plant nutrient and. soil suppressive capacity.

Conclusions

Results showed that biofertilizer application had the potential to increase functional diversity in soil microbial communities and soil microbial biomass. Actinomyces, soil suppressive capacity, glomalin and enzyme activity were strongly correlated. However it is important to make greater efforts to understand the behavior of a broad group of soil biological properties and how they relate to each other in particular areas and situations to contribute to generate information for more globally applicable evaluation of soil quality.

FEMS7-2018
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THOUGH INHABITANTS OF THE INTERNATIONAL SPACE STATION

M. Mora[1], A. Perras[1], L. Wink[1], T. Alekhova[2], R. Krause[1], C. Moissl-Eichinger[1,3]
[1]Medical University of Graz, Internal Medicine, Graz, Austria
[2]Moscow State University, Department for Microbiology, Moscow, Russia
[3]BioTechMed, -, Graz, Austria

Backgrounds

An important part of planning future long-term space flight missions (e.g. to Mars) is the development of strategies to safely manage the indoor microbiome of spacecraft, which represent completely isolated habitats. Since microorganisms pose a potential hazard for crew and spacecraft material, it is critical to assess microbial population dynamics and also to detect eventually developing resistances within the microbiota of such special closed systems. The best model system to date to investigate the microbiome of a confined habitat with constant human occupation in space is the International Space Station (ISS).

Objectives

To contribute to the big picture of the ISS indoor microbiome in addition to already existing data, we analysed 8-12 years old dust samples from Russian ISS modules with a focus on extremotolerent bacteria and archaea.

Methods

We assessed the cultivable microbiota of these desiccated samples via a broad range of cultivation assays and analysed their extremotolerant potential. Additionally, we assessed the microbial community of these samples via Next Generation Sequencing (NGS).

Conclusions

Besides confirming the presence of a broad variety of microorganisms onboard the ISS, of which many are equipped with a profound resistance capacity gainst clinically relevant antibiotics and other parameters, we were able to prove the presence of archaeal signatures on board the ISS. Elucidating the microbial population dynamics of the ISS and other similarly confined habitats can not only be used for spaceflight purposes but also help understanding possible risks in confined habitats on Earth, as e.g. industrial clean-rooms or confined hospital wards.

FEMS7-1628
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MICROBIAL COMMUNITY EVOLUTION IN ORGANIC FRACTION OF MUNICIPAL SOLID WASTE BY ANAEROBIC DIGESTION

S. Morandi[1], P. Cremonesi[2], T. Silvetti[1], C. Cortimiglia[2], M. Decimo[1], E. Casaletta[3], B. Castiglioni[2], M. Brasca[1]
[1]Institute of Sciences of Food Production ISPA-CNR, Italian National Research Council CNR, Milan, Italy
[2]Institute of Agricultural Biology and Biotechnology IBBA-CNR,
Italian National Research Council CNR, Lodi, Italy
[3]Austep SpA, Austep SpA, Milan, Italy

Backgrounds

The organic matter originated from the household waste treatment facilities has potential agronomic properties as fertilizer and soil amendment. On the other hands, digestate products could contain pathogens or otherwise harmful microorganisms such as aerobic and anaerobic spore-forming bacteria.

Objectives

This work aimed to investigate in different process plants the bacterial population of organic fraction of municipal solid waste (OFMSW) at the beginning and at the end of the digestion process using both conventional culture approach and metagenomics by Next Generation Sequencing (NGS). To deeply characterize the population of spore-forming bacteria in digestates, aerobic/anaerobic spore-forming bacteria were isolated.

Methods

Six biowaste samples of OFMSW and the corresponding digestates were analyzed for total viable count, *E. coli*, coliforms, enterococci, aerobic/anaerobic spore forming bacteria, yeasts and moulds. Spore-forming bacteria were isolated and identified by 16S rRNA sequencing. For metagenomics analysis, the bacterial DNA was extracted using a commercial kit and 16S rRNA gene amplicons on V3-V4 region analyzed by Miseq (Illumina).

Conclusions

Output digestates showed a good hygienic quality and lower counts of bacteria than OFMSW samples. Most abundant spore-forming bacteria belonged to *Bacillus aerophilus*, *B. amyloliquefaciens*, *B. cereus*, *B. licheniformis*, *B. safensis*, *B. subtilis* and *Clostridium sporogenes* species. The bacterial community of OFMSWs was dominated by *Firmicutes*, *Proteobacteria* and *Bacteriodetes*, while the output digestates revealed higher presence of *Synergistetes*, *Thermotogae* and *Euryarcheota*. The study provides new insights into microbiota evolution of OFMSW digestate and potential spores that can contaminate soils and crops when digestate is used as fertilizer

FEMS7-0860
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

COMPARISON OF TWO DIFFERENT PRIMER SETS AND AMPLIFICATION CONDITIONS USED IN AN 18S RRNA AMPLICON-BASED METAGENOMICS STUDY

L. Moreno-Mesonero[1], Y. Moreno[1], I. Amorós[1], M.A. Ferrús[2], J.L. Alonso[1]
[1]Instituto Universitario de Ingeniería del Agua y Medio Ambiente- IIAMA, Universitat Politècnica de València, Valencia, Spain
[2]Departamento de Biotecnología, Universitat Politècnica de València, Valencia, Spain

Backgrounds

Currently, molecular techniques are the most promising methods for the sensitive, accurate, and simultaneous detection of protozoan parasites. Among these, 18S rRNA amplicon-based metagenomics could be a powerful tool to investigate the presence of pathogenic protozoa all together in the environment.

Objectives

In this study, two different sets of designed primers, able to amplify simultaneously most of the protozoan organisms, were tested in a mock sample, sequenced by 18S rRNA metagenomics and analysed by an optimized bioinformatics pipeline.

Methods

The first set of primers was EUKAF 5´-GCCGCGGTAATTCCAGCTC-3´ and EUKA21R 5´-CYTTCGYYCTTGATTRA-3' and the second one was F18S-G: 5'-CGGCGGTAATTCCAGCTC-3' and R18S-G: 5'-TCYAAGAATTTCACCTCT-3'. An *in silico* PCR was conducted on SILVA databases allowing 2 mismatches (https://www.arb-silva.de/search/testprime/) for both sets of primers. Different enhancing agents for PCR, such as BSA, DMSO, glycerol, betaine or acetaine were tested, with the normally used PCR mix in order to improve Giardia spp. DNA amplication. Two polymerase systems, Accuprime G+C and KAPA HiFi with G+C buffer, were also tested. A mock sample containing *Cryptosporidium hominis*, *Giardia intestinalis*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Toxoplasma gondii* and *Blastocystis hominis* was amplified with both sets of primers using G+C KAPA HiFi buffer, sequenced in an Illumina MiSeq system and analysed in QIIME™.

Conclusions

The amplicon sequencing with the first primers and the use of the KAPA HiFi system with G+C buffer allowed the recovered of all the sequences from the mock, and although the second primers did not detect *Giardia* spp., they allowed a better identification of the rest of microoorganisms.

FEMS7-1405
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

PSEUDOMONAS FLUORESCENS SBW25 USES N-OCTANOYL-L-HOMOSERINE LACTONE AS A QUORUM SENSING MOLECULE.

O. Moshynets[1], S. Rogalsky[2], D. Irodov[1], S. Karakhim[3], S. Tarnavsky[4], M. Kharhota[5], G. Volynets[4], A. Spiers[6]
[1]Institute of Molecular Biology and Genetics- National Academy of Sciences of Ukraine, Cell Regulatory Mechanisms, Kiev, Ukraine
[2]Institute of Bioorganic Chemistry and Petrochemistry- National Academy of Sciences of Ukraine, Laboratory of Modification of Polymers, Kiev, Ukraine
[3]Palladin Institute of Biochemistry- National Academy of Sciences of Ukraine, Biochemistry of Muscle, Kiev, Ukraine
[4]Institute of Molecular Biology and Genetics- National Academy of Sciences of Ukraine, Medical Chemistry, Kiev, Ukraine
[5]Zabolotny Institute of Microbiology and Virology- National Academy of Sciences of Ukraine, Antibiotics, Kiev, Ukraine
[6]Abertay University, School of Science- Engineering and Technology, Dundee, United Kingdom

Backgrounds

Pseudomonas fluorescens SBW25 is a soil and plant-associated bacteria which form cellulose-based biofilms at the air-liquid interface of static microcosms. Robust biofilms are associated with mutations in diguanylate cyclases resulting in the induction of the cellulose synthase complex. However, it remains unclear whether biofilm formation, maturation and detachment involve quorum sensing (QS) regulation as is commonly observed for other pseudomonads.

Objectives

Objectives were assess a possible role of QS in *Pf.* SBW25.

Methods

Bioinformatics, cultivation assays, GC-MS and CLSM were used.

Conclusions

Our bioinformatics analysis of the *Pf.*SBW25 genome revealed a number of QS genes such as AHL synthase-like protein of HdtS family, LuxR-family proteins, and orthologues of the TpbA/TpbB system which control biofilm formation in *P. aeruginosa* PA01. In preliminary work we found that N-dodecanoyl-L-homoserine lactone (C12-HSL) influenced *Pf.*SBW25 biofilm structure, repressing cellulose production and increasing biofilm thickness. We were able to detect the presence of N-octanoyl-L-homoserine (C8-HSL) in *Pf.*SBW25 cultures grown in King's B medium by GC/MS, but could not detect it in PSM minimal medium cultures where less well-developed biofilms were also formed. The QS inhibitor azithromycin able to repress AHL production and biofilm formation in *Pa.*PA01 also repressed *Pf.*SBW25 biofilm formation without effecting growth, adding further evidence that AHL plays some sort of role in biofilm formation by SBW25. Our task for future work is to reveal the specific effects of C8-HSL in biofilm-forming and planktonic cultures, and to determine how QS and c-di-GMP regulation combine to regulate *Pf.*SBW25 biofilm formation in experimental microcosms and in natural environments.

FEMS7-2212
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

DETECTION OF BIOCONTROL RELATED GENES AND THE EFFECT OF VOLATILE COMPOUNDS OF PSEUDOMONAS ISOLATES ON THE MOBILITY OF XANTHOMONAS AXONOPODIS PV PHASEOLI

A. moura[1], M. Sangiogo[1], I. Souza-Junior[1], J. Pelegrineli[1], R. Moccellin[1]
[1]Universidade Federal de Pelotas, Fitossanidade, Pelotas, Brazil

Backgrounds

Pseudomonas spp. are widely recognized as biocontrol agents of diseases of different plant species. One of the main mechanisms of biocontrol of these bacteria is antibiosis, given the diversity of antimicrobial compounds produced by this group.

Objectives

The aim of this study was to detect genes related to the production of antimicrobial compounds and the influence of volatile compounds produced by biocontrollers on the motility of *Xanthomonas axonopodis* pv. *phaseoli* (XAP).

Methods

PCR was performed with primers specific for the genes *phlD* (2,4-diacetylphloroglucinol), *phzCD* (phenazine-1-carboxylic acid), *prn* (pyrrolnitrin), *plt* (pyoluteorin), *pupA* (pseudobactins), *gacA* (cyanide production), *hcnBC* (hydrogen cyanide), for the isolates DFs513 (*Pseudomonas veronii*), DFs831 (*P. fluorescens*) and DFs842 (*P. fluorescens*), previously selected for the control of XAP. In addition, an experiment was performed to evaluate the effect of volatile compounds on XAP motility.

Conclusions

The *phlD*, *phzCD* and *prn* genes were detected in the isolates DFs831 and DFs842, whereas the *phlD* gene was detected in all three isolates. In the PCR for the *gacA*, *plt* and *pupA* genes, none of the reactions were positive for the isolates in study. The isolates DFs831 and DFs842 reduced significantly XAP motility (25% and 40%, respectively). Thus, isolates DFs831 and DFs842 have a greater diversity of genes related to the production of antimicrobial compounds, and are still able to reduce the motility of XAP through the volatile compounds produced.

FEMS7-2823
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

TEMPERATURE IMPACTS THE BACTERIAL MOTILITY OF BIOCONTROLLERS SELECTED TO CONTROL DISEASES OF COMMON BEANS

A. moura[1], J. Fasolin[1], I. Souza-Junior[1], M. Sangiogo[1], R. Moccellin[1]
[1]Universidade Federal de Pelotas, Fitossanidade, Pelotas, Brazil

Backgrounds

The success of plant diseases biocontrol is dependent of the performance of the antagonistic microorganisms and the environment conditions where they are inserted. Currently the impact of climate changes on the behavior of microorganisms has been in foccus, since the increase of the ambient temperature can influence the microorganisms survival.

Objectives

In order to evaluate an influence of different temperatures on the ability of preselected biocontrollers bacteria in common bean crop, the present study evaluated in vitro swarming motility.

Methods

The experiment was conducted in a completely randomized design with five replicates. The isolates DFs93 and DFs769 (*Bacillus cereus*), DFs348 (*Bacillus* sp.), DFs513 (*Pseudomonas veronii*); DFs831 and DFs842 (*P. fluorescens*), DFs843 and DFs912 (*Rhodococcus fasciens*) were incubated at different temperatures: 17, 22, 27, 32 and 37 °C. The bacteria were grown in LB agar (0.6%) grown in BOD under different temperatures for 24 hours. The evaluation was done measuring the bacterial colony diameters.

Conclusions

Higher temperatures provided higher colony diameters for most of the isolates, except for DFs513. The bacteria of *Bacillus* genus showed higher motility at temperatures of 32 and 37 °C. For *Pseudomonas* and *Rhodococcus* genus, the best temperature was 32 °C. The DFs513 isolate had higher motility at 17 °C, although without differing from 22, 27 and 32 °C. The results suggest that the increase in temperature may increase the spread and future colonization capacity of these bacteria.

FEMS7-2437
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

UNDERSTANDING THE IMPACT OF PRODUCTION ANIMALS ON THE DISSEMINATION OF MCR-1

G. Moyano[1], J.F. Delgado-Blas[1], A. Hoefer[1], N. Montero[1], B. Gonzalez-Zorn[1]
[1]Complutense University of Madrid, Animal Health, Madrid, Spain

Backgrounds

During the last decade, colistin has been catagorized as one of the last resort agents to treat multidrugresistant bacteria. Contrary to its application in humans, colistin has been a widely used antibiotic in animal production. The discovery of the first mobilizable colistin resistance gene (*mcr-1*) greatly concerns the scientific community, especially with regards to animal production. Here we present the preliminary results of the EFFORT project pertaining to the prevalence of *mcr-1* throughout animal production.

Objectives

The main objective of this work is to study the prevalence of *mcr-1* in pig, poultry and turkey farms.

Methods

A total of 60 farms within the inclusion criteria were selected in Spain: 20 pig farms, 20 poultry farms and 20 turkey farms. At each location 25 individual faecal samples were taken and 10 *E. coli* per farm were independently isolated from different samples. The presence of the *mcr-1* gene was determined by PCR and the XbaI-PFGE profiles of the *mcr-1* positive strains were compared.

Conclusions

The high prevalence of *mcr-1* was detected not only in single epidemic clones but also in unrelated clones. This suggests that the dissemination of *mcr-1* is simultaneously caused by horizontal and vertical gene transfer. The presence of an identical clone in pig and poultry farms demonstrates the capacity of different *mcr-1* bearing clones to adapt to new hosts. These results show that production animals act as a reservoir for *mcr-1* and underline the importance of fighting antimicrobial resistance with a one health approach.

FEMS7-0721
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

THE PROTEOMIC RESPONSE OF PSEUDOMONAS PUTIDA KT2440 DURING POLYHYDROXYALKANOATES SYNTHESIS

J. Mozejko-Ciesielska[1], *A. Mostek*[2]
[1]*University of Warmia and Mazury in Olsztyn, Department of Microbiology, Olsztyn, Poland*
[2]*Institute of Animal Reproduction and Food Research, Department of Gametes and Embryo Biology, Olsztyn, Poland*

Backgrounds

Polyhydroxyalkanoates (PHAs) are bacterial polyesters that are accumulated as discrete granules and used as a storage material for carbon and reducing equivalents. Generally, PHAs are synthesised when a carbon source is present in excess and an essential nutrient such as nitrogen, phosphate or oxygen is available in limited concentrations. In spite of the potential of PHAs, their introduction to the world-wide market is currently limited due to their increased production cost compared to their synthetic alternatives. The PHAs synthesis process could be improved by a better understanding of how the metabolic networks that are responsible for mcl-PHAs synthesis respond to culture conditions and the type of substrate.

Objectives

The main aim of this study is to determine the influence of nutrients limitation on the global changes in the protein expression profile during PHAs synthesis by *Pseudomonas putida* KT2440.

Methods

Bioreactor experiments were conducted to determine the effect of different substrates and culture conditions on cell growth, PHAs content and proteome profile. PHAs were isolated and purified to determine their composition by gas chromatography. Two-dimensional polyacrylamide gel electrophoresis was applied to investigate the differences in the protein profile during biosynthesis process.

Conclusions

The obtained results enabled to identify proteins that are involved in the regulatory mechanisms of PHAs synthesis. The proteome analysis provided the information about the molecular basis of the physiological phenomena driving the PHAs accumulation inside bacterial cells that occur under various growth conditions. The study is a good starting point to gain insight into the PHAs accumulation machinery.

FEMS7-0132
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

SPECIES-SORTING AND MASS-TRANSFER PARADIGMS CONTROL MANAGED NATURAL METACOMMUNITIES

S. Günther[1], K. Faust[2], S. Müller[1]
[1]Helmholtz Centre for Environmental Research, Environmental Microbiology, Leipzig, Germany
[2]KU Leuven-, VIB Center for the Biology of Disease, Leuven, Belgium

Backgrounds

In a circular economy wastewater treatment plants (WWTP) do not only purify water but produce biogas or/and recover phosphorus. These tasks are performed by microbial communities at different reactor localities in a WWTP.

Objectives

To avoid frequent reactor failures ecological theories were tested to better understand and, as a result, control wastewater metacommunities.

Methods

A complex microbial system consisting of six different interconnected localities was thoroughly investigated at full-scale for over a year. The metacommunity concept originating from macro-ecology was used to uncover mechanisms of community assembly by observing microbial interrelationships in and between the different localities via correlation and network analysis. The individual-based observation approach was applied using high-throughput microbial community cytometry in addition to next generation sequencing.

Conclusions

We found robust α-diversity values for each of the six localities and high β-diversity values despite directed connectivity between localities, classifying for endpoint assembly of organisms in each locality. Endpoint characteristics were based on subcommunities with high cell numbers whereas those with lower cell numbers were involved in dispersal. Perturbation caused abiotic parameters to alter local community assembly with especially the rare cells announcing community restructuration processes. The mass-effect paradigm as part of the metacommunity concept was identified by an increase in inter-locality biotic correlations under perturbation which, however, did not unbalance the predominant species-sorting paradigm in the studied full-scale metacommunity.
Data as generated in this study might contribute to the development of individual based models for controlling managed multi-species natural systems in future (Environment. Microbiol. 2017 online).

FEMS7-0133
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

HIGH-RESOLUTION FLOW CYTOMETRY TO DETERMINE MICROBIOTA DYNAMIC COLITIS-ASSOCIATED CHANGES IN MOUSE FECES

J. Zimmermann[1,2], T. Hübschmann[3], H.D. Chang[2], S. Müller[3]
[1]University of Bern, Department of Clinical Research Gastroenterology, Bern, Switzerland
[2]German Rheumatism Research Center Berlin DRFZ, Cell Biology Group, Berlin, Germany
[3]Helmholtz Centre for Environmental Research, Environmental Microbiology, Leipzig, Germany

Backgrounds

The mammalian gut is colonized by a myriad of microbes referred to as the commensal microbiota. These bacteria are not only important for nutrient metabolism but also for the development and homeostasis of the immune system. Pathological changes in the composition of the microbiota, known as dysbiosis, are suggested to contribute to an array of diseases including inflammatory bowel disease, arthritis, encephalitis, and cancer.

Objectives

The profiling of the commensal microbiota by 16s rDNA or metagenome sequencing has become the current standard in the field, but it is time and labor intensive. Flow cytometry offers unique options to analyze the heterogeneity of the intestinal microbiota. We tested this cytometric approach for the profiling of the microbiota from healthy and colitic mice.

Methods

We introduce a method based on single cell light scattering and DNA content to analyze the heterogeneity and dynamic changes of the intestinal microbiota in the murine model of T-cell transfer-induced colitis.

Conclusions

We could demonstrate that overall microbial diversity decreases and that changes in the composition of the fecal microbiota in colitis coincide with weight loss and diarrhea. This analysis resolves up to 80 different populations per fecal microbiota. Subpopulations are phylogenetically homogeneous and their frequencies change dramatically when monitored in the course of murine T-cell transfer-induced colitis. High-resolution flow cytometry of the microbiota provides a fast and inexpensive tool for the analysis of the microbiota and offers the unique opportunity to isolate defined bacterial subpopulations for further molecular and functional analysis (European J Immunol 2016, 46: 1300-1303).

FEMS7-1936
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

CYANIDE PRODUCTION BY PREY BACTERIA PROTECTS THEM AGAINST PREDATION BY BDELLOVIBRIO BACTERIOVORUS

W. Mun[1], H. Kwon[1], H. Im[1], S.Y. Choi[1], A.K. Monnappa[1], R.J. Mitchell[1]
[1]Ulsan National Institute of Science and Technology UNIST, Life science, Ulsan, Republic of Korea

Backgrounds

Bdellovibrio bacteriovorus is obligate predatory bacterium which is prey upon numerous gram-negative bacteria and ubiquitous in terrestrial and aquatic environments

Objectives

Co-existence of prey and predator is wide spread among bacterial feeding microorganisms include *B. bacteriovorus.* Predation by *B. bacteriovorus* gives impact on structure of bacterial communities and many bacteria have evolved defense mechanism against predation. In this study, we show that extracellular secondary metabolite produced by the soil bacterium *Chromobacterium piscinae* functions as a defense strategy against predatory bacteria

Methods

We found that *C. piscinae* is not predated by *B. bacteriovorus* when cultivated in Dilute Nutrient Broth (DNB). However, *C. piscinae* can be predated on within a nutrient-free buffer, HEPES. We examined that spent-DNB media of *C. piscinae* significantly delays predation of *E. coli* MG1655 by *B. bacteriovorus*. We identified that hydrogen cyanide (HCN) is major inhibitory metabolite of *C. piscinae* spent-media by removing cyanide with purging and scavenging with Vitamin B12a. The concentration of cyanide of DNB spent-media is about 110uM, we examined that it show same level of inhibitory effect with KCN 110uM solution.

Conclusions

The results suggest that bacterial secondary metabolites, hydrogen cyanide, responsible for the inhibition of *B. bacteriovorus* predation and thus likely contribute to protect prey against predation.

FEMS7-2889
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MOLECULAR IDENTIFICATION OF CYANOBACTERIA IN A RESERVOIRS TRAIN LOCATED IN THE DEPARTMENT OF ANTIOQUIA, COLOMBIA

L.M. Múnera[1], N.J. Pino[1], G.A. Peñuela[1]
[1]University of Antioquia, GDCON Research Group- School of Microbiology, Medellin, Colombia

Backgrounds

Cyanobacteria are photosynthetic microorganisms that has been linked mainly to eutrophic waters. The excessive load of nutrients received by bodies of water has generated a considerable and worrisome increase of blooms around the world. These blooms are related with problems of public interest as toxicity, alteration of the food chain and its functionality, deoxygenation of the water column, reduction of biodiversity, among others.

Objectives

The objective of this study was to evaluate the relationship between the presence of cyanobacteria and the physicochemical variables: pH, temperature, TA, TP, DO, DOC, TOC, NTK, NO_3 and dissolved iron measured in three reservoirs located between 2.300 and 900 meters above sea level.

Methods

Sampling was carried out in the study reservoirs during the months of November of 2015, February, April and June of 2016. Within each reservoir, three sampling points were chosen: tributary entrance with higher flow, central area of the reservoir and exit close to catchment. The collected samples were evaluated for *in situ* and *ex situ* physicochemical parameters according to the procedures described by APHA, 2012.

Cyanobacterial samples were collected using a phytoplankton mesh. DNA extraction and amplification of the 16s rRNA gene using specific cyano-primers were performed. The PCR products were separated by DGGE, statistically analyzed using GelCompar® II program, bands were excised, purified and sent to sequencing. The edited sequences were analyzed on GenBank using BLAST.

Conclusions

This study concludes that the meteorological conditions and economic activities surrounding the reservoirs analyzed have a strong relation with the presence of cyanobacteria identified

FEMS7-2041
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part II

MECHANISMS OF BACTERIAL CONVERSION AND DEGRADATION OF PHARMA POLLUTANTS FROM NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

I. Ivshina[1], E. Tyumina[2], E. Vikhareva[3]
[1]Institute of Ecology and Genetics of Microorganisms UB RAS,
Laboratory of Alkanotrophic Microorganisms, Perm, Russia
[2]Perm State National Research University, Microbiology and Immunology, Perm, Russia
[3]Perm State Pharmaceutical Academy, Analytical Chemistry, Perm, Russia

Backgrounds

Bacterial degradation of pharma pollutants from non-steroidal anti-inflammatory drugs (NSAIDs, particularly diclofenac) is an effective method for their removal from contaminated environments by biocatalytic processes.

Objectives

1. To demonstrate the use of *Rhodococcus* to degrade diclofenac, the leading pollutant from NSAID group detected in the environment. 2. To study the interaction mechanisms of rhodococci and the pharma pollutant. 3. To investigate the major metabolic pathways for bacterial conversion of diclofenac, to identify metabolites, and to analyze several diclofenac-degrading enzymes.

Methods

The work was performed using the bioresources of IEGM Collection of Alkanotrophic Microorganisms (WDCM # 768, www.iegmcol). Chromatographic and mass-spectral methods were employed to detect diclofenac and its metabolites. A combined scanning system of an atomic force microscope and a confocal laser scanning microscope was used to study the specific features of actinobacterial interactions with the anthropogenic exotoxicant. Physiology of key bio-oxidizers was investigated by high-resolution respirometry.

Conclusions

For the first time the ability of *Rhodococcus* to efficiently biodegrade diclofenac in the presence of *n*-hexadecane was demonstrated. Using laboratory microcosms and model soil systems, novel basic data contributing to the in-depth insight into the processes occurring with pharma pollutants, the so-called “emerging contaminants”, in the environment were obtained.The developed heterogeneous biocatalysts based on *Rhodococcus* immobilized on solid carriers will be employed to design efficient biotechnologies for removal of pharma pollutants from waste water, and ecologically safe technologies for pharmaceutical waste detoxification and disposal.

The research was supported by the UB RAS Integrated Program and the Russian Foundation for Basic Research.

FEMS7-0408
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE PHYLOGENETIC CORE OF THE HUMAN PAN-MICROBIOME

D. Aguirre de Cárcer[1]
[1]*Unidad de Genética, Ciencias, Madrid, Spain*

Backgrounds

Whether or not there is a 'core' gut microbiome, consisting of bacterial groups common to all healthy humans, remains an open question with important scientific and medical implications. Unfortunately, the strong variability in gut microbiome composition persistently observed across individuals has so far hindered efforts towards its detection.

Objectives

The present study analyzes the human gut core pan-microbiome in terms of 16S rRNA OTUs present in all individuals, where such OTUs have been produced dynamically over a range of phylogenetic depths, as opposed to using an arbitrary fixed threshold which represents the dominant practice in the field.

Methods

This approach was applied in the meta-analysis of three large and comprehensive datasets (totaling 2,243 individuals; > 150M sequences).

Conclusions

The results show that the human gut pan-microbiome contains a preeminent compositional phylogenetic core, defined in terms of discrete units of varying depth along the bacterial phylogeny, whose members are present in all individuals studied.

This result provides a new conceptual framework with great potential for advancing our understanding of the ecosystem. In addition to providing a novel perspective on (i.e.) the study of community assembly, the results obtained in this study should guide the selection of more meaningful combinations of bacterial species (or genomes) in many frequent *in vivo*, *in vitro*, or *in silico* experimental scenarios. Furthermore, the results should be used as a revised list of "most wanted" bacteria to guide future genome sequencing and isolation efforts, significantly as it also includes information on the biologically meaningful breadth of the targeted group's pan-genome.

FEMS7-3167
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ECOLOGICAL INTERPLAYS IN THE TRANSITION TO MULTICELLULARITY

B. Baselga Cervera[1], *M. Travisano*[2]
[1]*Universidad Complutense de Madrid, Animal Production, MAdrid, Spain*
[2]*University of Minnesota, College of Biological Sciences, st. Paul, USA*

Backgrounds

Throughout the history of life, the evolution from single-celled to pluricellular organisms emerged, somehow serendipity, more than 20 times under separate scenarios. This mayor evolutionary transition constituted a source of novelty that reshaped the Earth's biodiversity and ecology. Notwithstanding recent discoveries both experimentally and in the fossil record, the underling processes and mechanisms of multicellularity arise are poorly understood.

Objectives

The complex interplays between organisms and their environments, lead to multiple interactions, features and properties. Thus, ecological conditions might have played a significant role during the transition to multicellularity and it persistence. Here, we examined the implications of different environmental conditions, related with resources availability, in the origin of multicellularity from an ecological perspective.

Methods

By means of experimental evolution and capitalizing the advantages of using the model organisms *Saccharomyces cerevisiae*, we have performed two different 2x2 factors experiments based on the original experiment of Ratcliff et al. (2012). Each experiment presented two types of environmental conditions: YPD rich media and Minimal media, and two types of treatment; no selection and settling selection, starting from differentness ancestral populations: a unicellular *S. cerevisiae* strain (Y55) and an evolved multicellular strain, snowflake yeast strain.

Conclusions

The evolution from unicellular to multicellular phenotypes took place in minimal media under setting selection, however we found different phenotypic distributions among replicates. The recently evolved snowflake yeast phenotype strain did not return to its ancestral unicellular phenotype in any of the environmental conditions. Ecological differences can lead to different results in the evolution of multicellularity.

FEMS7-1969
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COMPARISON OF METHODOLOGIES USED IN THE ANALYSIS OF ESCHERICHIA COLI IN TREATED SEWAGE SLUDGES

A. Lopez[1], M. Benito[1], J. Gomez[2], R. Mosteo[1], A.M. Lasheras[2], P. Goñi[3]
[1]Water Quality &Treatment Research Group-Institute of Environmental Science, Chemical Engineering and Environmental Technologies, Zaragoza, Spain
[2]NILSA, NILSA, Pamplona, Spain
[3]Parasitology- Self-care&Environmental Health Research Group, Preventive Medicine and Public Health, Zaragoza, Spain

Backgrounds

In wastewater treatment plants the microorganisms tend to concentrate in the sludge line and the control of pathogens has become an important issue on sludge management. There are current regulations for the disposal of biosolids for agricultural applications in USA and Europe being *Escherichia coli* considered a microbial indicator of sludge quality.

Objectives

The objective of this study is to compare different techniques based on standard analytical methodologies and using various culture media in the analysis of *E.coli* in two treated sludge samples (SM9221E, ISO72351:2005, SM9222D, SM9215B/C/D, UNE-EN-ISO16649-1/2/3). Specifically, membrane filtration (0.45µm), spread plate, pour plate and multiple tubes techniques were used.

Methods

Sludge samples used in the study came from a real wastewater treatment plant situated in Navarra(Spain). Raw sludge was treated in anaerobic digesters at 35°C/15days (mesophilic digestion) or at 55°C/10days (thermophilic digestion). Samples treated thermophilically presented lower microbial concentration than samples treated mesophilically. Assays were done in triplicate.

Conclusions

The results were different in function of the bacteria concentration in the sludge. When the *E.coli* concentration in treated sludge sample was≥10^3UFC/g there were not remarkable differences in terms of sensibility in the compared techniques and spread plate technique presented the results with the highest accuracy. On the other hand, when the *E.coli* concentration in treated sludge sample was≤10^3UFC/g, membrane filtration technique presented highest sensibility to quantify the bacteria in the majority of the culture media considered; the other techniques used show high uncertainty and low accuracy. The obtained results indicate that current regulations should be updated and similar criteria established.

FEMS7-2233
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PRESENCE OF POTENTIALLY PATHOGENIC PROTOZOA IN URBAN WASTEWATER TREATMENT PLANTS: RISKS OF REUSE

M. Benito[1,2], D. LaPlante[1], E. Rubio[1], A. López[2], N. Miguel[2], J. Gomez[3], A.M. Lasheras[3], M.P. Ormad[2], M.P. Goñi[1]
[1]University of Zaragoza, Parasitology- Self-Care and Environmental Health PSEH- DGA-FSE B124. Microbiology- Preventive Medicine and Public Health., Zaragoza, Spain
[2]University of Zaragoza, Water Quality and Treatment DGA-FSE T33: Environmental Sciences Institute of the University of Zaragoza . Chemical Engineering and Environmental Technologies, Zaragoza, Spain
[3]Navarra de Infraestructuras Locales S.A., I+D, Pamplona, Spain

Backgrounds

Reutilization of effluents and sludge from wastewater treatment plants(WWTP) provides a new application for this voluminous residue. However, the presence of potentially pathogenic protozoa may present a risk to public health. Current normative regulates the presence of *E. coli*, nematode eggs, *Legionella* spp., and *Salmonella* spp. Nevertheless, there are no references for protozoa, even though the use of traditional indicators does not predict their presence.

Objectives

The aim of this work is to study the presence of potentially pathogenic parasites and protozoa in water and sludge from WWTP with different treatments and evaluate the risk associated with their reutilization.

Methods

Five different WWTP located in the Ebro River basin(Spain) were studied. The presence of *Cryptosporidium* spp., *Giardia duodenalis*, and *Entamoeba* spp. was analysed using conventional methods and molecular biology techniques for genus and genotype identification. Free living amoebas(FLA) were cultivated and genetically identified. The presence of Amoeba Resistant Bacteria(ARB) inside FLA was also analysed.

Conclusions

Entamoeba was detected in all effluents, followed by *Cryptosporidium*(40%) and *Giardia*(20%). In sludge, *Cryptosporidium* was positive by PCR in all WWTP, *Entamoeba* spp. in 75%. *Giardia* was not detected. Forty-one FLA were isolated, 32 from water and 9 from sludge. Six FLA were indentified as *Naegleria* spp., 2 as *Vermamoeba* spp. and 13 as *Acanthamoeba* spp. ARB were detected in 27 of the FLA isolated, including *Legionella* spp. and *Mycobacterium* spp.

Persistance of potentially pathogenic protozoa has been observed in effluents and sludge, suggesting that more intensive treatments, than those currently employed, are needed in case of agricultural reuse.

FEMS7-1321
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CONSISTENT DYNAMICS AND COMMUNITY ASSEMBLY PATTERNS DIRECTLY ARISING FROM THERMODYNAMIC THEORY OF MICROBIAL GROWTH

H. Delattre[1]
[1]*IRSTEA, HBAN, antony, France*

Backgrounds

Microbial communities are key engines that drive earth biogeochemical cycles. Developing models able to capture and predict their dynamics and community assembly patterns is therefore of outmost importance for the study of global earth ecological equilibria and the development of innovative microbial biotechnology processes. However, ecosystem models today exhibit only limited abilities in predicting microbial dynamics and require the calibration of multiple population specific empirical equations.

Objectives

In order to build more generic models, there is a need to more thoroughly capture the fundamental drivers of microbial growth and to mathematically express how they contribute to the emergence of the many community assembly patterns observed in nature.

Methods

We simulate the dynamics of microbial functional communities using a kinetic model of microbial growth based on statistical physics (Desmond-Le Quéméner & Bouchez, 2014). We show how the theory coupled to simple mass and energy balance calculations offer a parsimonious explanation for many well-known microbial community assembly patterns along redox gradients.

Conclusions

Strikingly, considering only these simple physical assumptions, we show that consistent microbial dynamics, successions and functional community assembly patterns can be obtained, without population-specific parameter calibration, for systems ranging from pure cultures to communities of multiple populations.

FEMS7-1320
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EVALUATION OF METAGENOMIC METHODS FOR THE STUDY OF THE GUT MYCOBIOME

A. Frau[1], B. Campbell[1], A. Darby[2], J. Kenny[3], N. Hall[4], J. Anson[5], C. Probert[1]
[1]University of Liverpool, Institute of Translational Medicine- Cellular and Molecular Physiology, Liverpool, United Kingdom
[2]University of Liverpool, Institute of Integrative Biology- Functional and Comparative Genomics, Liverpool, United Kingdom
[3]University of Liverpool, Institute of Integrative Biology- Centre for Genomic Research, Liverpool, United Kingdom
[4]Earlham Institute, -, Norwich, United Kingdom
[5]Royal Liverpool University Hospital NHS, Infection and Immunity, Liverpool, United Kingdom

Backgrounds

Metagenomics has revolutionized the study of the gut microbiome but most methods are tailored for bacteria.

Objectives

The mycobiome likely plays a key role in intestinal diseases and hence metagenomic methods need to be adapted to study this. Here, we evaluated two DNA extraction methods and 5 primer sets.

Methods

Genomic DNA was extracted from seven characterised fungal cultures (*C. albicans*, *C. tropicalis*, *C. neoformans*, *M. furfur*, *S. cerevisiae*, *A. fumigatus* and *P. crysogenum*) using either the PSP Spin Stool DNA kit (Stratec), with and without extra bead-beating (6 min, 0.1 and 0.5 mm zirconia beads), or the QIAamp Fast DNA Stool Mini Kit (QIAGEN), and compared using 18S rRNA qPCR. Subsequently, stool was spiked with cells and spores from the same species and gDNA extracted (as above). DNA from spiked stool samples and a mock community were amplified and sequenced. Five primer sets were tested (18S rRNA, ITS1, ITS2-2X and 28S rRNA) for HiSeq Illumina sequencing.

Conclusions

Results: 1) the extra bead-beating did not increase the extraction; 2) the Stratec Kit extracted more gDNA from *C. albicans* VS *A. fumigatus* (Cp=29.1 SD=0.52 and Cp=31.9 SD=0.51), the reverse occurred with QIAGEN kit (Cp=34.3 SD=0.74 and Cp=26.6 SD=0.06); 3) on stool DNA similar amounts of 18S rRNA copies/ng of DNA were observed with both kits. Sequencing data are being currently evaluated: this will show how extraction methods and primer sets influence analysis of the mycobiome. In conclusion, the method of DNA extraction and primer design influence results and interpretations of the mycobiome.

FEMS7-0896
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CONTRIBUTION OF THE PSEUDOMONAS FLUORESCENS MFE01 TYPE VI SECRETION SYSTEM TO BIOFILM FORMATION

M. Gallique[1], *V. Decoin*[1], *C. Barbey*[1], *A. Merieau*[1]
[1]*Laboratoire de Microbiologie Signaux et Microenvironnement EA 4312, Université de Rouen- Normandy University, EVREUX, France*

Backgrounds

To persist in an ecological niche, bacteria have developed several strategies to enable them to resist in the environment. One of these mechanisms, able to struggle with other bacterial species, is the Type VI Secretion System (T6SS). This allows bacteria to inject toxins directly into prey cell cytoplasm. A mucoid environmental isolate of *Pseudomonas fluorescens,* the MFE01 strain, has antibacterial activity carried by T6SS [1]. Indeed, MFE01 can immobilise prey cells through the action of the Hcp1 protein of the T6SS [2]. Currently, genomic analysis reveals only one T6SS core component cluster and three orphan *hcp* genes (named *hcp1*, *hcp2* and *hcp3)*.

Objectives

Aims of this study are to assign a role to each Hcp proteins (Hcp1, Hcp2 and Hcp3) in biofilm maturation and competition against prey strains during co-inoculation in biofilm condition.

Methods

Biofilms were grown on a glass surface, for 48 h at 28°C, under a flow of LB medium and observed by confocal laser scanning microscopy. Prey cells bearing pSMC2.1 *gfp*, encoding green fluorescent protein, were co-cultured alone or with MFE01 or derivatives, in a 1:5 ratio.

Conclusions

Mutations of *hcp1*, *hcp2* and *hcp3* did not reduce *P. fluorescens* MFE01 biofilm formation [3], but the three Hcp proteins were required for the completion of biofilm maturation. Moreover, a mutant with a disruption of one of the unique core component genes, MFE01Δ*tssC*, was unable to produce its own biofilm or to inhibit prey cells biofilm formation.

FEMS7-1297
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

HOW DO MARINE BACTERIA INTERACT WITH THE AMOEBA ACANTHAMOEBA CASTELLANII ?

R. Guillonneau[1], *C. Baraquet*[1], *M. Molmeret*[1]
[1]*Université de Toulon, MAPIEM EA 4323, La Garde, France*

Backgrounds

Amoebae are common in soils, freshwater and marine ecosystems. They are suspected to have an important role in most microbial ecosystems, especially in biofilm. However the interaction between these protozoa and marine bacteria is poorly investigated

Objectives

The aim of this work is first to study interactions between different marine bacteria and the axenical amoeba *Acanthamoeba castellanii* in order to identify different behavioural patterns. The second objective is to understand if, in this context, the bacterial biofilms could provide any sort of advantage against this predator.

Methods

Bacteria-specific antibodies were used during the co-culture experiments for confocal laser scanning microscopy observations while amoebas were labelled with DAPI. Transmission electronic microscopy was also used to visualize intracellular localisation of bacteria.

Conclusions

These marine bacteria presented different profiles of interaction with *Acanthamoeba castellanii.* They appeared to be localized in different intracellular compartments or to behave in a singular manner. For instance, *Persicivirga mediterranea* (TC4) was located in the amoeba nucleus, which could at least in part protects the bacteria from phagolysosomal digestion. *Shewanella sp.* (TC11) appears to be within suspected expelled fecal balls after its interaction with the amoeba. The objective was to identify the different bacterial behaviour within the host cell, and to understand if these behaviours could trigger specific outcomes when the protozoa is added to monospecific and multispecies biofilms. This is one of the first study comparing different isolated marine bacterial species in presence of the amoeba *A. castellanii*.

FEMS7-1811
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECT OF CUSO4 AND ZN2SO4 ON TRANSFER OF ANTIBIOTIC RESISTANCE GENES PROFILES FROM ENVIRONMENTAL BACTERIA TO ESCHERICHIA COLI

C.J. Guzman Otazo[1], V. Iniguez[2], J. Larsson[3], Å. Sjöling[1]
[1]Karolinska Institutet, Microbiology- Tumor and Cell Biology MTC, Stockholm, Sweden
[2]Universidad Mayor de San Andres UMSA, Institute of Molecular Biology and Biotechnology, La Paz, Bolivia
[3]University of Gothenburg, Department of Infectious Diseases- Institute of Biomedicine, Gothenburg, Sweden

Backgrounds

The increase of antibiotic resistance genes (ARG) in pathogenic bacteria is considered one of the biggest threats for human health worldwide. Environmental compartments might be important reservoirs of antibiotic resistance genes (ARGs) and where transfer and spread of environmental ARGs to human-associated bacteria needs to be studied. The presence of metals in the environment has been associated with selection of antibiotic resistance in bacteria by means of cross and co-resistance mechanisms.

Objectives

In this study, we evaluated the impact of addition of two different metal salts: $CuSO_4$ and Zn_2SO_4 on lab mating experiments using environmental bacteria as donors and the lab *Escherichia coli* strain CV601 as recipient.

Methods

Donors were obtained from two different sampling points in the highly polluted Choqueyapu River in La Paz, Bolivia and prepared for mating conditions without any prior culture, thereby including also the non-cultivable bacterial fraction. Two different concentrations of metal salts (0,5M and 1M) were evaluated for its effects on Transfer Frequency (TF) using Sulfamethoxazol 150 ug/ml supplemented plates as transconjugants selection media.

Conclusions

Our results showed that the there is no significantly increase in Transfer Frequency (TF) after 3 hours of mating in media with metals salts versus control LB media. Different antibiotic resistance patterns will be analyzed by Kirby-Bauer Disk Susceptibility Test in the > 300 transconjugants recovered from the mating experiments in order to identify the potential association between co-selection of metals and the type of antibiotic resistance pattern transferred.

FEMS7-2724
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE MICROBIAL ECOSYSTEM OF THE GREENLAND ICE SHEET SURFACE

C.S. Jacobsen[1], K. Cameron[2], S. Marek[3]
[1]Aarhus University, Environmental Sciences, Roskilde, Denmark
[2]Geological Survey of Denmark and Greenland, Department of Geochemistry, Copenhagen, Denmark
[3]Charles University, Department of Ecology, Pragh, Czech Republic

Backgrounds

The surface of the Greenland ice sheet (GrIS) harbors a simple yet unique microbial ecosystem. The biological active margin of the ice sheet is more than 200 000 km^2, can be up to ~100km wide, and has tended to increase in area each year within recent history. This ecosystem contains microbial communities that are important for biogeochemical processes and surface melt and that are likely important for the injection of biota and nutrients into downstream environments.

Objectives

Our research suggests that the GrIS surface receives a “baseline” cell supply via deposition of atmospheric waters, and that wind-borne dust deposited on the ice sheet likely contains additional cells and may provide limiting nutrients for microbial growth. Ablation areas with high dust concentrations and longer melt seasons are therefore expected to contain higher numbers of active microbes compared to the accumulation area.

Methods

Geochemical anlysis, qPCR and amplicon seqiencing.

Conclusions

Within the ablation area, significant differences exist between the bulk and potentially active bacterial communities collected at the margin and the interior of the GrIS, with a higher total abundance but lower proportion of active bacteria at the margin. Higher microbial activity in the interior compared to the margin is also supported by productivity measurements. Nitrate had no significant effect on the abundance and community structure, which suggests that this system is not nitrate limited. In terms of diversity, we do not find a core, uniform bacterial community across the biologically active area of the GrIS; instead, km-scale variations between the microbial communities exist.

FEMS7-3321
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

AUTOREGULATED BIOTOPE SUPPORTED BY SYMBIOTIC SYSTEMS RECOGNIZING POLYVALENT POLYMERIC GLYCOCONJUGATES

M. Lakhtin[1], V. Lakhtin[1], S. Afanasiev[1], A. Bajrakova[1], V. Aleshkin[1]
[1]Medical Biotechnology, G.N.Gabrichevsky Institute of Epidemiology and Microbiology, Moscow, Russia

Background
Microbial communities participate in biotope healthy status.
Objectives
The objective was to develop one more strategy of supporting healthy human biotope.
Methods
Probiotic systems recognizing glycoconjugate patterns **(**PSRGP) of human probiotic bacteria; intestinal and urogenital microbial clinical strains were used. Microbes were grown in standard media and conditions in the absence or presence of PSRGP.
Conclusions
1. *Features of* PSRGP. PSRGP are localized, organized and act as cell layer mosaic surface and secreted truncated/ modified and assembled systems imitating pro/syn/symbiotics. Molecular-cellular PSRGP are represented by synergistically cofunctioning probiotic microorganisms (autoregulated probiotic pool of strains producing bifidobacterial and lactobacillar lectins, their complexes, protective glycosyl hydrolases and oxydoreductases). PSRGP function as major and minor soluble and surface signals directed against virulent factors. PSRGP reveal features of metabolomebiotics, net switchers, quorum sensing molecules, cross-talking cells. PSRGP reveal synergistic cascade antipathogen (antifungal, antistaphylococcal) effects towards ranging targets. **2.** *Biotope communications. L.acidophilus* and *L.casei* selected strains limit fungal growth of *C.albicans, C.tropicalis, C.krusei* or *C.albicans+A.niger* as well as influence subspecies microecological niches distribution within group of increased infection risk (*C.albicans* and *C.tropicalis*). *L.acidophilus* and *L.casei* PSRGP include leader strains which interrupt communications between *Candida* species pools or within *C.albicans* continuous massive. PSRGP participate in on duty elimination of changeable potentially dysbiotic lactobacillar strains. On the other hand, biotope *Candida* pools contain leader strains which interrupt communications between probiotic (*Lactobacillus*) species. **3.** *Strategy of supporting biotope*: screening probiotic and potentially pathogenic leader strains; delivery of synbiotic PSRGP; elimination of antibiotics-sensitive potentially pathogenic leader strains. **4.** Conception of biotope multilevel-healthy net-knot multitaxonomic microbiocenose is proposed.

FEMS7-2494
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DISINFECTION BYPRODUCT-INDUCED TRANSFORMATION OF COLLOIDAL ANTIBIOTIC RESISTANCE GENES (ARGS) IN NATURALLY COMPETENT BACTERIA

D. Mantilla[1]
[1]*King Abdullah University of Science & Technology KAUST, BESE, Thuwal Jeddah, Saudi Arabia*

Backgrounds

Disinfection of the treated wastewater leads to the formation of disinfection byproducts (DBPs), which are genotoxic agents formed due to the reaction of chlorine with naturally occurring organic matter. The genotoxicity of these compounds rely on their ability to cause double-strand breaks in DNA of viable cells. In prokaryotes, double-strand breaks are repaired by diverse mechanisms including homologous recombination mediated by RecA. This protein is also involved in other cellular processes such as the integration of DNA into the bacterial genome, which constitutes a critical step in the mechanism of natural transformation. In addition, reclaimed wastewater has shown to carry high abundances of antibiotic resistance genes (ARGs), which could be partially found as extracellular DNA in the colloidal fraction of such treated effluents. Due to the presence of extracellular ARGs and the central role of RecA in DNA repair and transformation, we hypothesize that DBP-mediated DNA damage may enhance foreign DNA integration in naturally competent bacteria.

Objectives

The objective of this project is to evaluate whether exposure to DBPs might increase the fixation of ARGs using *Acinetobacter baylyi* as a model, a naturally competent and environmentally ubiquitous microorganism.

Methods

To evaluate transformation rates, a reporter strain of *A. baylyi* that expresses a green fluorescent protein and spectinomycin resistance as a result of a transformation event was used. Transformation rates were initially evaluated in cell cultures exposed 0, 75, 150 and 200 uM of bromoacetic acid (BAA), a known mutagen and regulated DBP.

Conclusions

Transformation rates of the donor DNA increased at the higher BAA concentrations following a dose response curve. Besides spectinomycin resistance, transformants were further confirmed by PCR to ensure the presence of the donor DNA.

FEMS7-2243
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL METAGENOMIC AND METATAXONOMIC STUDY OF BILIARY SAMPLES IN PATIENTS WITH CHOLEOLITHIASIS AND A CONTROL GROUP

N. Molinero[1], I. Gutierrez-Diaz[2], C. Milani[3], M. Mangifesta[3], C.M. García-Bernardo[4], A. Cabrera[5], J.I. Rodríguez[5], S. Gonzalez[6], M. Ventura[3], A. Margolles[1], S. Delgado[1]
[1]IPLA-CSIC, Microbiology and Biochemistry of Dairy Products, Villaviciosa, Spain
[2]University of Oviedo, Functional Biotogy, Oviedo, Spain
[3]University of Parma, Life Sciences, Parma, Italy
[4]HUCA, Cirugía General, Oviedo, Spain
[5]Hospital de Cabueñes, Servicio de Cirugía General y del Aparato Digestivo, Gijón, Spain
[6]University of Oviedo, Functional Biology, Oviedo, Spain

Backgrounds

In recent years, the relationship between cholesterol, bile salt metabolism, intestinal microbiota and its potential implication in health is been elucidated. However, the composition and role of the biliary microbiota remains obscure. The characterization of the human bile microbiota as a whole has been hampered by difficulties in accessing biological samples and the lack of adequate methodologies to assess molecular studies.

Objectives

The aim of this work was to generate new knowledge of human bile microbial profiles, functions and activities in patients with choleolithiasis vs healthy individuals, and identify possible dysbiosis associated with this pathology.

Methods

To characterize the biliary microbiome of patients with cholelithiasis and healthy subjects we collected bile samples from 15 gallstone patients and a comparable control group. We performed with Illumina technology high throughput sequencing of 16S rDNA amplicons, together with whole metagenome shotgun of selected bile samples.

Conclusions

A high diversity was present in the biliary microecosystem, with main fila represented: *Firmicutes*, *Bacteriodetes*, *Actinobacteria* y *Proteobacteria*. Significant differences in the relative abundance of different taxa present in the bile of both groups were found. Sequences belonging to the family *Propionibacteriaceae* were more abundant in bile samples from healthy subjects, meanwhile in patients with choleolithiasis members of the families *Bacteroidaceae, Prevotellaceae* y *Veillonellaceae* were more frequently detected.

Sequencing of total DNA from bile of three healthy subjects and functional analysis allowed us to observe that the main COG functions and categories were similar to that describe in the human intestinal microbiome.

FEMS7-1428
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION AND CHARACTERIZATION OF AN ALKANES-DEGRADING PSEUDOMONAS AERUGINOSA FROM GULF OF MEXICO

L.F. Muriel-Millan[1], J. Rodríguez-Mejía[1], D. Xahonero-Canavesi[1], W. Escobedo-Hinojosa[1], A. Estradas-Romero[1], H. Sánchez-Texis[1], L. Pardo-López[1]
[1]Institute of Biotechnology-UNAM, Gulf of México Research Consortium CIGoM, Cuernavaca, Mexico

Backgrounds

Oil spills have severely affected different marine ecosystems due to its high persistence in the environment. Alkanes represent an important fraction of total hydrocarbons that conform the crude oil and its biodegradation is a challenge for different microorganisms due to their low solubility and reactivity. Nevertheless, some bacteria have the metabolism to synthesize surfactants that permit the alkane solubilization and their posterior degradation.

Objectives

The aim of this study was to characterize the hydrocarbon-degrading capacity of a strain isolated from Gulf of México.

Methods

Initially, a bacterial consortium from water column (55 meters of depth) was isolated from Gulf of México in mineral medium with a mix of oil crude and kerosene as carbon and energy source. From this consortium, a strain named B9MF-1 was isolated and phylogenetic analysis of 16S rRNA sequence showed that this strain is closely related to *Pseudomonas aeruginosa* species. B9MF-1 strain was able to grow in mineral medium using hexadecane (C16) or eicosane (C20) as sole carbon source. Microbial Adherence To Hydrocarbon (MATH) assays showed that, cells previously adapted in medium with hexadecane, had a cell surface hydrophobicity of 70%, while in presence of glucose, the strain exhibited a hydrophobicity of 37%. Analysis of the cell culture supernatants by thin-layer chromatography, revealed the presence of aminolipids and glycolipids in cultures added with hexadecane but not in those added with glucose.

Conclusions

These results indicate that cell surface properties and synthesis of glycolipids and aminolipids are associated to the capacity of *P. aeruginosa* B9MF-1 strain to degrade aliphatic hydrocarbons.

FEMS7-2165
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EXAMINING THE INFLUENCE OF MODE OF DELIVERY ON THE GUT MICROBIOTA COMPOSITION IN ADULTHOOD

A.B. Murphy[1,2,3], P.J. Kennedy[2,4], J.F. Cryan[2,5], R.P. Ross[1,2], P.W. O'Toole[2,3], T.G. Dinan[2,4], C. Stanton[1,2]
[1]Teagasc Food Research Centre, Food Biosciences Department, Cork, Ireland
[2]University College Cork, APC Microbiome Institute, Cork, Ireland
[3]University College Cork, Microbiology Department, Cork, Ireland
[4]University College Cork, Department of Psychiatry, Cork, Ireland
[5]University College Cork, Department of Anatomy & Neuroscience, Cork, Ireland

Backgrounds

Colonisation of the infant gut early in life has an important role in directing development of the immune system, nervous system and brain. It is well established that birth by Caesarean (C)-section disrupts the normal colonisation of the infant gut due to the lack of contact between the new-born and maternal vaginal and intestinal microbes. Indeed, there is accumulating evidence that C-section delivery can have many long lasting health effects including the development of immune and metabolic disorders.

Objectives

The objective of this study was to examine the long term impact of mode of delivery on the gut microbiota composition of young adults.

Methods

The effects of C-section birth were investigated by examining the gut microbiota of a cohort of male adults aged between 18 and 24, either born vaginally (N=36) or by C-section (N=31). Participants did not differ in age, body mass index (BMI) or units of alcohol consumed per week. Data were also collected on general gut health, diet and physical activity. The gut microbiota of both groups was profiled using 16S rRNA gene amplicon sequencing.

Conclusions

The microbiota of C-section and vaginally-born subjects showed similar pair-wise relative abundances at phylum level with Firmicutes and Bacteroidetes dominating. This was reflected at family and genus levels with few significant differences present between participants. Additionally, diversity levels were not influenced by mode of delivery. These findings indicate that the gut microbiota disturbances due to C-section which occur during infancy do not persist to adulthood.

FEMS7-1882
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COMPLEX MICROBIAL PREPARATION FOR REMEDIATION OF SOIL MICROBIAL CENOSES AND PROMOTION OF CROP PRODUCTIVITY

T. Nasonava[1], M. Mandryk-Litvinkovich[1], L. Kartyzhova[2], Z. Aleschenkova[2], E. Kolomiets[1]
[1]Institute of Microbiology - Belarus National Academy of Sciences,
Laboratory of Biological Control Agents, Minsk, Belarus
[2]Institute of Microbiology - Belarus National Academy of Sciences,
Laboratory of interactions between soil microorganisms and higher plants, Minsk, Belarus

Backgrounds

Analysis of contemporary state of agricultural production, evaluation of dynamics of land qualitative parameters highlight the tendency of falling soil fertility and aggravation of general ecological situation in agroindustrial sector. Intensive mechanical and chemical treatment of arable fields, inadequate supply of organic nutrients coupled to erosion processes lead to irreversible loss of soil bioorganic potential. As a result the structure of microbial associations undergoes changes affecting steady-state of soil ecosystem, its ability to sustain fertility and productivity. One of the ways to decrease pressure on biocenoses is formulation and use in agrotechnologies of effective biopreparations derived from living microbial cultures.

Objectives

The study was aimed at composing microbial preparation for restitution of soil microbial cenosis and increase of crop harvests.

Methods

Microbiological, biochemical, molecular-genetical methods were engaged in this research.

Conclusions

Based on 5 bacterial strains representing genera *Bacillus*, *Pseudomonas*, *Brevibacillus*, a complex microbial preparation displaying a number of valuable properties was developed:

- it suppresses development of pathogenic microbiota and ensures recovery of agrobiocenoses;
- accelerates degradation of plant residues in soil;
- mobilizes insoluble phosphorus compounds;
- enriches soil with biological nitrogen;
- upgrades soil fertility;
- raises productivity of cultivars by 10-20%.

Application of microbial preparation allows to replace supply of chemical and mineral fertilizers, to increase crop harvests and to yield eco-friendly products at minimal costs.

FEMS7-0956
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION AND IDENTIFICATION OF GAMM RRADIATION RESISTANT BACTERIA FROM LOUT DESERT OF IRAN

A. nasrollahi omran[1], M. Mohseni[2], J. Abbaszadeh[1]
[1]Faculty of biology Sciences- Islamic Azad University of Tonekabon Branch- Tonekabon- Iran, Department of Microbiolgy, Tonekabon, Iran
[2]Department of Molecular and Cell Biology- University of Mazandaran- Babolsar- Iran, Department of Molecular and Cell Biology- University of Mazandaran- Babolsar- Iran, babolsar, Iran

Backgrounds

Members of genus Deinococcus are able to live in extreme conditions such as arid deserts, under ionizing radiations, ROS (reactive oxygen species) molecules and other oxidative stress inducing chemicals.

Objectives

Aim of this study is to investigate microorganisms that live in surface soil of desert where is exposed to high temperatures at days and low temperature at nights, also desiccation and rehydration. The Lout desert of Iran isknown for its high surface temperature that is named as the hottest surface place on earth. Both ionizing radiation and desiccationmay cause damage on genome

Methods

Two native ionizing radiation-resistant bacteria were isolated and identified from a soil sample collected from extreme conditions of the Lout desert in IranSoil sample was irradiated in order to eliminate sensitive bacteria then cultured in onetenth-strength tryptic soy broth medium. Bacterial suspension used for radiation treatment. Morphological and physiological characterization and phylogenetic studies based on 16S rRNA gene sequence were used for identification.

Conclusions

The cells were rod shape, non-motile, non-spore forming and gram positive. The 16S rRNA gene sequence showed 99.5 % of similarity to Deinococcus ficus. Phylogenetic dendrogram demonstrated that the isolates branched with D. xibeiensis, D. ficus and D. mumbaiensis. Phylogenetic analysis showed the relationship between LD4 and LD5 isolates and other Deinococcus species.Both isolates were resistant to>15 kGy of gamma radiation and >600 J m^{-2} of UV radiation. This is the first report on radiation resistant bacteria belonging to genus Deinococcus isolated from the Lout desert of Iran.

FEMS7-0974
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EVATUATION OF DRUG SUSCEPTIBILITY OF ANTIFUNGAL DRUGS AGAINST ASPERGILLUS SPECIES ISOLATED FROM ICU OF HOSPITALS AT INVITRO

A. nasrollahi omran[1]
[1]*Faculty of biology sciences- Tonekabon Branch -Islamic Azad University- Tonekabon -Iran-, Department of Microbiology, tonekabon, Iran*

Backgrounds

Invasive aspergillosis is a disease that could threaten the patients having immune system defects which causing nosocomial fungal infections and high mortality in them.There are some resons including delayed diagnosis, lack of appropriate curing, and exsisting the various diseases and neutropenia which lead to the high mortality rate of patients with aspergillosis, especially in paiteients of intensive care units (ICUs).

Objectives

The aim of this study was to assess the sensitivity of the antifungal drug on Aspergillus sp. isolated from the hospital

Methods

After collecting 160 plates containing SDA medium from the air of hospital's ICU and incubating, the suspected fungal colonies grown on macroscopic and microscopic morphology were approximately detected. DNA extraction and molecule identification were performed using sequencing method with specific primers. Ultimately the species identification was fulfilled using microdilution method and drug susceptibility tested for existing drugs using CLSIM38A2 guidance for isolates in 96 microplates

Conclusions

According to a study of 40 plates suspected to *Aspergillus*, containing 11 definitive colonies after sequencing, 5 cases were *A. flavus*, 3 cases *A. sydowii*, 1 case *A. fumigatus* and 2 cases were confirmed as *A. Oryzae*. Regarding to susceptibility test, *A. sydowii* and *A. fumigatus* were sensitive to Amphotericin and Voriconazole and were sensitive to Itraconazole. *A. sydowii* was resistant to Caspofungin while *A.* fumigatus was sensitive to the drug. Also *A. flavus* was sensitive to all study drugs.

FEMS7-2122
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

FABRICATING RECHARGABLE N-HALAMINE NANOPARTICLES FOR ANTIFOULING APPLICATIONS

M. natan[1]
[1]*bar ilan university, life sciences, ramat gan, Israel*

Backgrounds

Biofilm formation is a serious problem in medical and industrial settings due to the increased resistance of these communities to killing compared to free-living bacteria. This has prompted the search for agents that can inhibit both bacterial growth and biofilm formation. In this study, N-halamine rechargeable nanoparticles (NPs) were synthesized by co-polymerization of the monomer methacrylamide and the cross-linker monomer N,N-methylenebisacrylamide, and were subsequently loaded with Cl^+, using bleach. The chlorinated NPs demonstrated remarkable stability and durability to organic reagents and to repetitive bacterial loading cycles. The antibacterial mechanism of the P(MAA-MBAA)-Cl NPs involved generation of reactive oxygen species (ROS) only upon exposure to organic media, but not upon suspension in water, revealing that the mode of action is target-specific. Further, a unique and specific interaction of the chlorinated NPs with *Staphylococcus aureus* bacteria but not with human cells was discovered, whereby these microorganisms were all specifically targeted and marked for destruction. Finally, in collaboration with Netafim Ltd. irrigation drippers containing the P(MAA-MBAA)-Cl were incubated in the field and were shown to prevent fouling on them for 5 months compared with the control, hence providing the drippers with 'self-cleaning' and 'self-sterilizing' properties. Further, the NPs offer recharging to the surface, thus providing long-lasting protection that does not exist in the products available today. In summary, our findings underscore the potential of developing sustainable P(MAA-MBAA)-Cl NPs-based devices for inhibiting bacterial colonization and growth.

Objectives

synthesize rechargable n-halamine nanoparticles

Methods

synthesizing nanoparticles by co polymerization process

Conclusions

n-halamine nanoparticles were synthesized to carry halogens and proved to be effectvie against microorganisms

FEMS7-1239
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

AMENDMENT WITH HIGH DOSES OF AEROBIC AND ANAEROBIC BIOSOLIDS PRODUCED LONG-TERM CHANGES IN THE METABOLIC PROFILE AND THE BACTERIAL COMPOSITION OF A CROP SOIL

F. Navarro-García[1], I. Nadal-Rocamora[1], C. Gondim-Porto[1], L. Platero[1]
[1]Dept. Microbiología II- School of Pharmacy- Universidad Complutense de Madrid, Microbiología II, Madrid, Spain

Backgrounds

The use of biosolids as amendments is a frequent way to increase the fertility in crop soils as well as to give added value to a residue whose amount is raising every year due to national regulations. However, the effect of these compounds at high rates needs to be assessed more deeply in order to determine the long term modifications in the soil microbiota that could hamper the normal function of crop soils under Mediterranean climate.

Objectives

To evaluate the effect of two types of high doses of biosolids on the bacterial population composition and their metabolic profiles of a crop soil in a 2-year experiment.

Methods

Crop soils amended with 160 $Tm \cdot ha^{-1}$ of an aerobic and an anaerobic biosolid were analyzed every four months for two years. The metabolic profiles of microbial populations (CLPPs) were obtained using the Microresp© system. The composition of bacterial populations was determined by pyrosequencing 98 16S rDNA libraries. Sequences were prepared and taxonomically asigned using Mothur, VAMPS and Greengenes. This work has been financed by projects CGL2006-13915/CLI and MMA022/PC08/3-04.2.

Conclusions

The proportion of *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Gemmatimonadetes* and *Verrucomicrobia* phyla increased, while *Acidobacteria*, *Cyanobacteria* and *Planctomycetes* phyla decreased upon treatment, producing the anaerobic biosolid a largest number of microbial changes that allowed us to define a set of relevant microbial markers. Nevertheless, the effect of amendment on CLPPs was similar, a result that could be explained by a functional redundancy of bacterial populations. Several microbial groups were statistically related with the metabolism of some chemical compounds.

FEMS7-2549
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

AQUATIC FUNGI AND THEIR SURFACE COUNTERPARTS IN THE HAINICH CRITICAL ZONE EXPLORATORY

A. Nawaz[1,2], *F. Buscot*[1,3], *T. Wubet*[1,3]
[1]*Helmholtz Centre for Environmental Research, Department of Soil Ecology, Halle Saale, Germany*
[2]*University of Leipzig, Department of Biology II, Leipzig, Germany*
[3]*German Centre for Integrative Biodiversity Research, iDiv, Leipzig, Germany*

Backgrounds

Subsurface groundwater aquifers are primarily recharged by infiltration of surface water, which brings down not only dissolved organic matter but also the surface microbial communities into the subsurface. Aboveground surface conditions like vegetation cover and landuse management types have profound impact on the belowground fungal community structures and compositions. Whereas the effects of these surface conditions on subsurface fungal communities beyond the rooting zone in fresh water aquifers at different depths are largely unknown

Objectives

Objective of this study were to:
- investigate the fungal community structures in two subsurface groundwater aquifers and their surface recharge areas
- find the dominant functional groups in surface and subsurface
- find the core fungal microbiome of surface and subsurface and their shared taxa

Methods

Soil and water samples were analyzed by using DNA based high-throughput sequencing of fungal internal transcribed spacer (ITS) rDNA gene to compare the fungal community structure and composition in two habitats.

Conclusions

Our results indicated that the fungal OTUs belonging to phylum Basidiomycota and Ascomycota dominate the aquifers. The core fungal microbiome of surface and subsurface samples indicates the high similarities between two habitats reflecting that surface conditions have impact on the subsurface fungal communities. Specifically the presence of different functional groups and, in particular plant and cattle pathogens that are not typical of subsurface habitats, further strengthened that their potential transport of fungi between surface and subsurface. In addition to this, we also find fungal taxa specific to the subsurface samples only and that points to a groundwater specific fungal microbiome.

FEMS7-0655
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIODEGRADATION OF CHLORPYRIFOS BY MICROBES ISOLATED FROM RICE PADDY FIELDS: EFFECT OF BIOSTIMULATION AND IMPACT OF MIXED MICROBIAL CONSORTIA

T. Nayak[1], *T.K. Adhya*[1], *M. Suar*[1], *V. Raina*[1]
[1]*KIIT University, KIIT School of Biotechnology, Bhubaneswar, India*

Backgrounds

Chlorpyriphos (CP) is a broad-spectrum organophosphorous pesticides (OP) majorly used for pest (termites, beetles) control in agri-crops like rice, maize, soyabean, cauliflower etc in many countries. Extensive use of this compounds result in their accumulation in agricultural soil, water source and affecting insects and humans by inhibiting acetylcholinesterase in the nervous system. Degradation of CP results in the formation of a compound TCP (3,5,6-trichloro-2-pyridinol) classified as mobile, toxic also has antimicrobial property.

Objectives

Present investigation showed (a) bacterial strains isolated and screened for their degradation ability from agricultural soil amended with CP and (b) study the effect of biostimulation on CP degradation using mixed microbial consortia.

Methods

Resting cell assay for Chlorpyrifos degradation in liquid media followed by HPLC analysis and biostimulation using carrier material for Chlorpyrifos degradation.

Conclusions

Strains *Ochrobactrum* CPD-03, *Microbacterium* CPD-20 and *Bacillus* CPD-33 were identified based on 16s rRNA approach. Effect of biostimulation on the CP degradation in stimulated (addition of nutrients) and non-stimulated conditions were performed resulting 88% CP degradation in stimulated condition and 66% degradation in non-stimulated condition respectively. This showed CP degradation under stimulated condition has provided nutritional environment for CP degradation. Corncob acted as a good carrier material for CPD-03 viability resulted enhanced CP degradation. It was also found that, the consortia MC-1 had shown better degradation efficiency in liquid media (80% degradation efficiency in 24 hours) and in presence of carrier material (58-60% degradation efficiency in 10 days). This can be potential for *in situ* bioremediation of contaminated soil ecosystem.

FEMS7-1779
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL COMMUNITIES CHARACTERISTICS DURING TWO-YEAR GROWTH OF BIOFUEL CROP MISCANTHUS X GIGANTEUS ON THE MILITARY CONTAMINATED SOIL

D. Nebeská[1], V. Pidlisnyuk[1], J. Trögl[1], L. Seidlová[1], P. Veronesi-Dáňová[1], P. Kuráň[1], L. Erickson[2], L. Davis[3]
[1]Jan Evangelista Purkyně University in Ústí nad Labem - Faculty of the Environmen, Department of technical sciences, Ústí nad Labem, Czech Republic
[2]Kansas State University, Department of chemical engineering, Manhattan, USA
[3]Kansas State University, Department of biochemistry & molecular biophysics, Manhattan, USA

Backgrounds

Phytoremediation of contaminated soils using second generation biofuel crops is a promising way to restore soil functions together with biomass production.

Objectives

Perennial grass *Miscanthus x gigantheus* was tested for cleaning metal (Ti, Fe, Mn, Zn, Sr, Zr) contaminated soil from post-military site in Sliač, Slovakia in two year pot experiment. Soil microbial community characteristics in rhizosphere and bulk soil were assessed by determination of phospholipid fatty acids (PLFA), respiration and activities of enzymes representing C, N, P, S cycles.

Methods

PLFA were determined according to ISO 29843-2. Enzyme activities were determined by direct incubation of soil with artificial enzyme substrates.

Conclusions

Microbial characteristics of polluted soil were lower in comparison to other agricultural soils. Significantly lower value of *cy/pre* PLFA stress indicator in rhizosphere soil compared to bulk soil and year-over-year comparison of activities of phosphatases, arylsulphatases, oxidases and soil respiration indicate gradual positive effect of *Miscanthus x giganteus* roots to soil microorganism.

FEMS7-1486
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

POLYPHASIC CHARACTERIZATION OF METHANOTROPHIC COMMUNITIES ENRICHED FROM ACIDIC SOILS

N.L. Nguyen[1], W.J. Yu[1], H.Y. Yang[1], J.H. Gwak[1], S.K. Rhee[1]
[1]Chungbuk National University, Department of Microbiology, Cheongju, Republic of Korea

Backgrounds

Acidic soil covers approximately 30% soils of the world and acidification is accelerated in agricultural soils. However, little is known about methane-oxidizing bacteria in acidic soil environments.

Objectives

We investigated the methanotrophic communities enriched from acidic soils using the sequencing batch reactor at pH 4 and isolated the acidophilic methanotrophs from enrichment culture.

Methods

Illumina sequencing for 16S rRNA gene analysis indicates that the families *Methylocystaceae* (15.3%), *Xanthomonadaceae* (10.0%), *Acidobacteriaceae* (8.7%), *Rhodocyclaceae* (5.0%), *Crenothrichaceae* (4.6%) and *Beijerinckiaceae* (0.7%) were major members in the methanotrophic communities. The family *Methylocytaceae*, *Crenothrichaceae* and *Beijerinckiaceae* might be key members involved in methane oxidation in acidic soils. The floating culture and extinction culture techniques were performed to isolate the key methanotrophic strains from the enrichment culture. Two isolates belonging to the genus *Methylovirgula* of the familiy *Beijerinckiaceae* and the genus *Methylobacter* of the family *Methylococcaceae* were obtained from the enrichment cultures.

Conclusions

Our findings suggest that various methanotrophs in diverse phylogenetic clades are involved in methane oxidation in acidic soils. Metagenomic sequencing for reconstruction of genome and their metabolic pathways of the dominant members in the acidic methanotrophic enrichments is on-going to get insight on the methane oxidation in acidic soils.

FEMS7-2451
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

REAL TIME ANALYSIS OF THE INTERACTION OF ISOLATED INTESTINAL MICROBIOTA AND SUPERNATANTS FROM DIFFERENT HUMAN POPULATION GROUPS WITH A COLON ADENOCARCINOMA CELL LINE

A.M. Nogacka[1], E. Gómez[1], G. Solís[2], M. Suárez[2], N. Fernández[3], A. Suárez[4], C. Martínez-Faedo[5], P. Ruas-Madiedo[1], N. Salazar[1], C. G. de los Reyes-Gavilán[1], M. Gueimonde[1]
[1]IPLA-CSIC, Microbiology and Biochemistry of Diary Products, Villaviciosa-Asturias, Spain
[2]Hospital Universitario Central de Asturias- SESPA, Pediatrics Service, Oviedo, Spain
[3]Hospital de Cabueñes, Pediatrics Service, Gijón, Spain
[4]Hospital Universitario Central de Asturias, Gastroenterology, Oviedo, Spain
[5]Hospital Universitario Central de Asturias, Endocrinology, Oviedo, Spain

Backgrounds

The gut microbiota constitutes a complex ecosystem that changes through the lifespan. Our understanding on the functional characterisitcs of the microbiota is mainly based on *in vitro* models that focus in the analysis of the interaction between the host and specific bacterial strains. Therefore, the development of simple and economically affordable study models, which take into account the complexity and functionality of the intestinal microbiota is required.

Objectives

The ability of an *in vitro* model, based on the real-time monitoring of the HT29 colon adenocarcinoma cell line, was evaluated to asses its ability to detect differences in the functionality of faecal supernatants (FS) and isolated faecal microbiotas (M) from different human population groups: obese (n = 9), healthy normal weight adults (n = 7) and ulcerative colitis patients (n = 5). A group of infants (n = 20), divided in full-term infants (2 days of age, n = 10) and preterm infants of the same age (n = 10) were also studied.

Methods

The composition of the M was characterized by 16S rRNA gene sequencing. The quantification of short chain fatty acids was also determined by gas chromatography in the FS. Moreover, the effect of M and FS was monitored in real time, using RT-Cell Analyzer technology on the proliferation and confluence phases of the HT29 cell line.

Conclusions

The microbiota-host interaction *in vitro* model developed allowed to detect differences in the functionality of the microbiotas of different population groups. This *in vitro* model shows potentiality for the characterization of the functionality of complex microbiotas and the resulting effect of modulating them by probiotics, prebiotics or other functional components of diet.

FEMS7-1243
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ACTIVITY OF BACTERIAL ENDOPHYTES ASSOCIATED WITH RICE UNDER ALUMINIUM TOXICITY

F. Nongkhlaw[1], *D. Thakuria*[1]
[1]*College of PG Studies - Central Agricultural University, NRM, Shillong, India*

Backgrounds

The decline in rice (*Oryza sativa* L.) production is responsible for enormous economic loss world-wide. The production of rice in the North-eastern part of India is limited by aluminium toxicity. Endophytes are microbes that reside inside tissue of healthy plants without causing damage. Endophytes have several beneficial effects on their host plants, including growth promoting activity, modulation of plant metabolism and phytohormone signalling. Endophytes play a vital role by synthesizing a wide range of bioactive metabolites that leads to adaptation to environmental stresses.

Objectives

This study involves the use of endophytic bacteria associated with *Oryza sativa* L. for agricultural applications and to ensure improved crop performance under contaminated soil conditions, such as Al toxicity. The study includes an investigation on the bioactive potential of bacterial endophytes under stressed environment.

Methods

Molecular characterization of bacterial endophytic communities living inside rice tissue grown in tissue culture at increasing concentration of aluminium (50-500 ppm Al). Screening of phytochemical compounds produced by endophytes associated with tissue cultured rice at increasing concentration of aluminium using gas chromatography-mass spectroscopy (GC-MS).

Conclusions

Rice tissue culture was successful in growing rice at increasing concentration of aluminium. Different bacterial populations were detected in culturable medium. The associated endophytes were observed to enhance the growth of rice under Al toxicity.

FEMS7-0098
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

LACCASE ACTIVITY CHANGE IN CRYPHONECTRIA HIPOVIRUS 1-INFECTED CRYPHONECTRIA PARASITICA IS INFLUENCED BY CULTURE CONDITIONS, FUNGAL ISOLATE AND VIRUS STRAIN

L. Nuskern[1], M. Tkalec[2], M. Gačar[1], M. Ježić[1], M. Ćurković-Perica[1]
[1]University of Zagreb- Faculty of Science, Department of Microbiology, Zagreb, Croatia
[2]University of Zagreb- Faculty of Science, Department of Botany, Zagreb, Croatia

Backgrounds

In chestnut blight fungus *Cryphonectria parasitica,* laccase, a polyphenol oxidase, is thought to be involved in pathogenesis towards chestnut due to its ability to degrade lignin, which is intensively synthetized by chestnut trees as a defensive reaction. Infection with virus *Cryphonectria hypovirus 1* (CHV1), a hyperparasite of *C. parasitica*, has been shown to reduce laccase activity of infected fungus, reducing its virulence towards chestnut. Thus far the research has been done on only limited number of fungal isolates and virus strains.

Objectives

Our aim was to determine the effect of local CHV1 strains on laccase activity in different Croatian *C. parasitica* isolates, which could have important implications in choosing the optimal virus strain for human aided biocontrol of chestnut blight.

Methods

Three CHV1 strains were transferred to three Croatian isolates of *C. parasitica* by hyphal anastomosis. The cultures were grown on potato dextrose agar and broth, and laccase activity was assayed with 2,2'-azino-bis(3-etilbenzotiazoline-6-sulphonic acid) as a substrate. For cultures grown on solid medium total laccase activity was measured, and for cultures grown in liquid medium extra- and intracellular laccase activity was measured.

Conclusions

When grown on solid medium, total laccase activity of CHV1-infected fungal isolates increased compared to control, virus-free isolates. In liquid medium extracellular laccase activity either increased or decreased, depending on fungal isolate, while the intensity of the change depended on the virus strain. Intracellular laccase activity of isolates grown in liquid medium showed almost no responsiveness to CHV1 infection. Thus we conclude that *C. parasitica* laccase activity depends on more parameters than just the CHV1 infection.

FEMS7-2870
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

16S RRNA SEQUENCING OF THE VAGINAL MICROBIOME IN BACTERIAL VAGINOSIS AND AEROBIC VAGINITIS

E. Oerlemans[1], S. Wuyts[1], S. Wittouck[1], I. Claes[1], G. Donders[2], S. Lebeer[1]
[1]UAntwerpen, Bioscience engineering, Antwerp, Belgium
[2]University Hospital of Antwerp, Gynaecology and Obstetrics, Antwerp, Belgium

Backgrounds

In most women, the healthy vaginal microbial community is characterized by a low complexity and often a dominance of *Lactobacillus* species, which exert an apparent protective role. In some medical conditions, these lactobacilli disappear. Bacterial vaginosis (BV) is recognized as an overgrowth of mainly anaerobic bacteria such as *Gardnerella vaginalis*, *Prevotella spp.*, *Atopobium vaginae* and *Mobiluncus* species. In contrast, aerobic vaginitis (AV) is less known, but culture and microscopy suggest an increased presence of more aerobic bacteria such as *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli* and Enterococci. Moreover, AV is mostly associated with an increased inflammatory response. Currently, AV is often misdiagnosed as BV, which might act as a confounding factor for studies investigating the microbiome in BV.

Objectives

Microbiome profiles of both AV and BV will be useful to gain more insight into these conditions, and help clinicians in the search for proper treatments.

Methods

We characterized the vaginal microbiome of 51 women, including subjects with a normal *Lactobacillus*-dominated microbiota (NL) and patients suffering from AV or BV. DNA was isolated from cervicovaginal lavage fluid, and used for *16S rRNA* amplicon sequencing of the V4 region using Illumina Miseq. The obtained data was analysed using the dada2 bioinformatic pipeline.

Conclusions

Our results indicate a more diverse community in BV as compared to the NL and AV group. Additionally, a unique *Gardnerella* ribosomal sequence variant was almost solely found in the BV samples. Contrary to the culture data, we found heterogeneous communities in the AV group, with mainly anaerobic species.

FEMS7-3159
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EXOCYCLIC DNA METHYLTRANSFERASES FROM MARINE ALPHABACTERIA

H.M. Oh[1], J. Kim[1], K.K. Kwon[2]
[1]Marine Genomics Lab, Marine Bio-Convergence, Busan, Republic of Korea
[2]Korean Institute of Ocean Science and Technology, Marine Biotechnology Research Division, Ansan, Republic of Korea

Backgrounds

DNA methylation is involved in a diversity of processes in bacteria, including maintenance of genome integrity and regulation of gene expression. CcrM, the DNA methyltransferase conserved in Alphaproteobacterial species, has N6-Adenine or N4-cytosine methyltransferase activities using S-adenosyl methionine as a co-substrate. Using PacBioRSII genomic data, methylation patterns were observed and summarized for a exocyclic DNA methylases of potential CpG and GpC methylation.

Objectives

Single molecule real-time sequencing method (SMRT) is a tool for genomic analysis as well as a methods to monitor bacterial DNA methylome. Here we report two distinct exocyclic DNA methyltransferase genes from two alphaproteobacteria strain from marine environments.

Methods

Using PacBioRS II sequencing, methylation patterns of *Celeribacter marinus* IMCC12053 and *Novosphingobium pentaromativorans* US6-1 were compared using Gibbs motif sampler program. Both strains have been observed to change adenosine of 5'-GANTC-3' as N6-methyladenosine, and N4-cytosine of 5'-CpG-3' (IMCC12053) and 5'-GpC-3' (US6-1) as N4-methylcytosine. Using phylogenetic analysis exocylic DNA methyltransferases from both of the species were chosen for cloning.

Conclusions

In this study cloned exocyclic DNA methylases are presented, and the potential use of novel type of CpG and GpC methylases in molecular biology and epigenetics.

FEMS7-3160
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COMPLETE GENOME ANALYSIS OF FLAVOBACTERIALES BACTERIUM STRAIN UJ101

H.M. Oh[1], J. Kim[1], K.K. Kwon[2]
[1]Marine Genomics Lab, Marine Bio-Convergence, Busan, Republic of Korea
[2]Korea Institute of Ocean Science & Technology, Marine Biotechnology Research Division, Ansan, Republic of Korea

Backgrounds

Flavobacteriales bacterium strain UJ101was isolated from a xanthid crab shell collected from East Sea of Korea. Here we report the complete genome sequence of strain UJ101 for the study of metabolic interaction between UJ101 and its host organism.

Objectives

Completed genome of strain UJ101 was compaired to other Flavobacteriales genomes for further analysis.

Methods

Single molecule real-time technology (PacBio RSII) was used for the single circular chromosome that is 3,074,209 base pairs in length and the GC content was 30.74%. The genome of UJ101 contains 2,698 ORFs with 46 tRNAs and 9 rRNAs genes. Genomic data were processed by the EzCG comparative genomic pipeline by www.chunlab.com with some customizations.

Conclusions

According to the annotated list of genes Embden–Meyerhof and pentose phosphate pathway is well conserved, but key enzymes of Entner-Douddoroff pathway were impared. TCA and glyoxylate cycle were conserved while carbon fixation and one carbon metabolism were mostly lacking except formaldehyde dehydrogenase. UJ101 encodes degradation enzyme including 8 glycosyl transferases, 3 amylases, and 8 peptidases. Biosynthetic enzymes for canonical amino acids were all conserved. Alcohol and/or organic acid fermentation could not be expected. Genomes from Flavobacteriales and related groups were chosen for comparative genomic analysis. Strain UJ101 was compared with bacterial genomes isolated from other marine animals (3 strains from invertebrate and 5 strains from fishes). Other related genomes from the same genera were included although they were isolated from seawater and marine sediments. UJ101 is deficient in biotin metabolism, which was also observed between marine fish dwelling isolates and seawater genomes in this study.

FEMS7-0263
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PREVALENCE OF SALMONELLA AND CAMPYLOBACTER IN CAPTIVE CROWS (CORVUS CORONE AND C. MACRORHYNCHOS) IN THE NORTHEASTERN JAPAN

M. Okamura[1], H. Sano[1], K. Sakata[1], M. Kaneko[1], J. Shindo[2], H. Shirafuji[3], D.L. Hu[1]
[1]Kitasato Univ. School of Veterinary Medicine, Laboratory of Zoonoses, Towada, Japan
[2]Kitasato Univ. School of Veterinary Medicine, Laboratory of Wildlife Sciences, Towada, Japan
[3]Kyusyu Research Station National Institute of Animal Health,
Subtropical Disease Control Unit Division of Transboundary Animal Diseases, Kagoshima, Japan

Backgrounds

Salmonella and *Campylobacter* cause human foodborne enteritis via consumption of raw/undercooked contaminated poultry meat and products worldwide to date. Broiler flocks are primarily contaminated with the bacteria. The pre-harvest control measures include the avoidance of transmission of the bacteria from environmental reservoirs to broiler flocks. Although the reservoirs have not clearly been identified, the roles of wildlife in the contamination of broilers are suggested recently.

Objectives

In the current study, we investigated the prevalence of *Salmonella* and *Campylobacter* in free-living crows to estimate the potential risk in contamination of broiler flocks with *Salmonella* and *Campylobacter*.

Methods

A total of 123 crows were captured between October 2012 and April 2014 in the box traps, and cloacal swabs were sampled for isolation of *Salmonella* and *Campylobacter*. Relatedness or diversity among the isolates were analyzed by pulsed field gel electrophoresis (PFGE).

Conclusions

Salmonella serovers Bredeney and Derby were isolated from only 11 of 123 crows (8.9%), which are not common in broiler chickens. *Campylobacter* spp. were isolated from all of 89 crows tested (100%) of which *C. jejuni* was the most prevalent (85 crows). PFGE patterns showed the vast diversity in *C. jejuni* isolates from crows and only possible relatedness to the broiler isolates epidemiologically. These results suggest the potential risk of transmission of *Salmonella* and *C. jejuni* from crows to broilers is limited.

FEMS7-0221
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COMPREHENSIVE ANALYSIS OF THE FECAL MICROBIOTA OF HEALTHY JAPANESE ADULTS REVEALS A NEW BACTERIAL LINEAGE ASSOCIATED WITH HIGHLY FREQUENT BOWEL MOVEMENTS AND A LEAN BODY-TYPE

K. OKI[1], M. Toyama[2], T. Banno[2], O. Chonan[1], Y. Benno[2], K. Watanabe[1]
[1]Yakult Honsha, Central Institute, Kunitachi Tokyo, Japan
[2]RIKEN Innovation Center, Benno Laboratory, Wako Saitama, Japan

Backgrounds

In Japan, a variety of traditional dietary habits and daily routines have developed in many regions. The effects of these behaviors, and the regional differences in the composition of the gut microbiota, are yet to be sufficiently studied.

Objectives

We aimed at characterizing the Japanese gut microbiota and identifying the factors shaping its composition.

Methods

We collected fecal samples from 516 healthy Japanese adults (325 females, 191 males; age, 21–88) residing in various regions of Japan and conducted 16S metagenomics analyses. Each participant also completed a 94-question lifestyle questionnaire.

Conclusions

Clustering analysis based on the fecal bacterial family composition showed that two enterotype-like clusters were observed in the males, but not the females, suggesting that the composition of the fecal microbiota has a gender-specific component. However, subjects' region of residence and gender were not strongly correlated with the general composition of the fecal microbiota.
The bacterial compositions were then compared with lifestyle questionnaire scores. The abundances of *Christensenellaceae*, *Mogibacteriaceae* and *Rikenellaceae* were negatively correlated ($P < 0.001$) with bowel movement frequency. Furthermore, the abundance of these bacterial families was significantly ($P < 0.01$ or 0.05) higher in lean subjects (BMI < 25) than in obese subjects (BMI > 30). The abundances of these families were positively correlated with each other and comprised a correlative network with 14 other bacterial families. These results implied that the abundances of *Christensenellaceae*, *Mogibacteriaceae* and *Rikenellaceae* contributed subject's highly frequent bowel movements and a lean body-type together with those of some other bacterial components comprising correlative network with them.

FEMS7-0015
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SPATIAL DISTRIBUTION AND DIVERSITY OF BACTERIAL ASSEMBLAGES IN THE LAURENTIAN GREAT LAKES: COMPARISONS BETWEEN LAKES MICHIGAN, ERIE AND HURON

O. Olapade[1]
[1]*Albion College, Biology & the Center for Sustainability & the Environment, Albion, USA*

Backgrounds

The Laurentian Great Lakes, including Lakes Superior, Michigan, Huron, Erie and Ontario, located in the eastern part of North America are considered the largest of freshwater lakes in the world. However, the diversity and distribution of indigenous microbial assemblages within these vast bodies of freshwater systems remains largely unexplored.

Objectives

To qualitatively and quantitatively delineate the microbial structure and community composition in these vast body of freshwater systems

Methods

Combinations of high-throughput sequencing of the 16S rRNA genes and fluorescent *in situ* hybridization (FISH) approaches were utilized to quantitatively characterize the occurrence, diversity and distribution of bacterioplankton assemblages in six different sites located along the coastal regions of Lakes Michigan, Huron and Erie.

Conclusions

Phylogenetic examination showed a diverse bacterial community belonging to eleven different taxonomic groups. Pyrosequencing results revealed that the majority of the sequences were clustered into four main groups i.e. *Proteobacteria, Bacteriodetes* and *Cyanobacteria*; while fluorescent *in situ* hybridization also showed the numerical dominance of members of the *GammaProteobacteria* and the *Cytopahaga-Flavobacteria-Bacteroidetes* (CFB) cluster in the six lake sites examined. Overall, the assemblages were shown to be quite similar in distribution among the lake sites examined, comprising of heterotrophic populations, except for the Sandusky Bay site in Lake Erie that was mostly autotrophic, with more than 50% of the population comprised by members of the Cyanobacteria. This further indicate that combinations of factors, including water chemistry, various anthropogenic disturbances and lake morphometric characteristics, are probably continuously influencing the community structure and diversity of the lake's bacterial assemblages.

FEMS7-2892
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DIVERSITY OF MOBILOME PRESENT IN TIETE RIVER

L.R. Olchanheski[1], L. Rada[1], E.G. Silva[1], S. Ichiwaki[1], F.L. Melo[1], W.L. Araújo[1]
[1]Biomedical Sciences Institute, Microbiology Department, São Paulo, Brazil

Backgrounds

Tiete river is the most important river in São Paulo State, and now a days is consider one of the most polluted river in the country. A factor that confer resistance and evolutionary advantages of determined organisms is the horizontal gene transfer (HGT). These advantages keep some organisms even in unfavorable conditions.

Objectives

For these reasons, the aim of this work were access the cover of genes with capacity of horizontal gene transfer in Tiete River.

Methods

Four sites were choose, based to their water quality index (very poor, fair, good and excellent). Metagenomic Shotgun sequencing of water samples were perform using Illumina HiSeq 2000™ platform. The assembly were perform using IDBA-UD. Transposase genes were finding by IsSaga2, and the genes nearby were annotated using the Metagenomics Rapid Annotation Server (MG-RAST).

Conclusions

The results revealed a distribution pattern of antibiotics resistance genes. In the site of very poor quality water, there were a prevalence of *sul*2 gene, which confer resistance to sulfonamide specifically. In contrast, in the good site the gene *sme*B (an efflux bomb) were prevalent. The results based only in the genes near insertion sequence, there is a prevalence of genes related to resistance to antibiotics and toxic compounds, mainly efflux bomb. Tiete River is a polluted river, receiving daily dumping of domestic and industrial sewage. These conditions keep some advantages to bacteria that contains the genes of antibiotic resistance.

Others genes can growth the fitness of bacteria in and impacted environmental, for this reason another genes near insertion sequence will be related with physical-chemical parameters and analyze if there is a distribution pattern of the genes that condition the bacteria fitness.

FEMS7-1161
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECTS OF LONG-TERM STARVATION ON VIBRIO HARVEYI MEMBRANE SUBPROTEOME DURING ITS PERMANENCE IN SEAWATER

M. Orruño[1], C. Parada[2], Z. Bravo[2], E. Ogayar[2], I. Larrañaga[2], V. Kaberdin[3], I. Arana[2]
[1]Faculty of Pharmacy - University of the Basque Country UPV/EHU, Immunology- Microbiology and Parasitology, Vitoria-Gasteiz, Spain
[2]Faculty of Science and Technology - University of the Basque Country UPV/EHU, Immunology- Microbiology and Parasitology, Leioa, Spain
[3]Faculty of Science and Technology - University of the Basque Country UP/EHU / IKERBASQUE- Ba sque Foundation for Science, Immunology- Microbiology and Parasitology, Leioa, Spain

Backgrounds

The behavior of *Vibrio harveyi,* pathogen for a wide range of marine animals, in environments is conditioned, among others, by temperature, salinity or nutrient scarcity.

Objectives

To evaluate the physiological and proteomic changes that take place during *V. harveyi* survival in seawater, especially those associated with the long-term survival.

Methods

V. harveyi populations were maintained for 230 days in sterile natural seawater at 20°C. The total and active cells were regularly monitored via epifluorescence microscopy, while the culturable bacteria were determined by the spread plate method in Marine Agar. Additionally, samples were collected for extraction of membrane proteins and their subsequent identification and quantification via mass spectrometry.

Conclusions

Nutrient-limited conditions led to the loss of *V. harveyi* culturability and its entry into the viable but nonculturable (VBNC) state after 14-15 days. The membrane subproteome composition also underwent changes during the survival process. Although the level of many proteins remained nearly the same after 3 weeks of starvation (initial phase), there was a fast disappearance of proteins involved in chemotaxis. The depletion of these non-essential proteins could prevent unnecessary waste of energy required for survival. In contrast to the initial phase, the long-term survival process (230 days) led to more profound changes in the membrane subproteome. While several proteins with structural and bioenergetics functions became undetected, a number of transport-related proteins increased their abundance.
Thus, in response to suboptimal conditions, *V. harveyi* triggered an adaptation process which included its entry into the VBNC state and the membrane subproteome reorganization.

FEMS7-1663
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECT OF HABITAT DISTURBANCES ON THE POPULATION DYNAMICS OF ALLOCHTHON LISTERIA MONOCYTOGENES IN SOIL

A.R. Ortiz Camargo[1], A. SPOR[2], S. GABA[3], D. Garmyn[4], L. Gal[5], P. Piveteau[4]
[1]UNIV. BOURGOGNE-FRANCHE-COMTE- UFR SANTE, Agroecologie, DIJON, France
[2]INRA, INRA UMR1347 Agroecologie, Dijon, France
[3]INRA, INRA UMR1347, Dijon, France
[4]UNIV. BOURGOGNE-FRANCHE-COMTE, INRA UMR1347 Agroecologie, Dijon, France
[5]AgroSup, INRA UMR1347 Agroecologie, Dijon, France

Backgrounds

Soil is in many cases the first stage in the routes of transmission of foodborne pathogens to plant, farm animals, foodstuff and humans as final consumers. Soil is a complex, heterogeneous environment which shelters many organisms such as allochthon bacteria. The persistence of these organisms depends on abiotic factors (temperature, humidity, texture, chemistry) and on biotic interactions, for example competition with native microflora.

Objectives

In this study, we used the foodborne pathogen *Listeria monocytogenes* as model system to investigate how disturbances affect habitat invasion by allochthon organisms.

Methods

Two soils with contrasting abiotic and microbiome characteristics were used. Inoculated soil microcosms were submitted to two cycles of temperature shifts (either increase to 42°C or freezing at -20°C) separated by 20 days of incubation at 20°C. Control microcosms were kept at 20°C. In order to investigate the impact of the indigenous microbiota, similar experiments were run in γ-irradiated, sterilized soil microcosms. Cultivable *L. monocytogenes* were evaluated by plate counts throughout the 40 days incubation. Additionally, soil samples were taken at the start of the experiment and after 20 and 40 days for DNA extraction and subsequent 16SrDNA diversity analysis. Variations of diversity were assessed in non-inoculated microcosms to evaluate the influence of *L. monocytogenes* invasion on the native soil microbiota.

Conclusions

While growth was observed in sterilized soil, the population of *L. monocytogenes* decreased in the other experimental conditions. Results showed that the fate of allochthon *L. monocytogenes* depended on the disturbance regimen. Higher survival was observed in when soil underwent cycles of freezing

FEMS7-1162
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ENVIRONMENTAL AND GEOGRAPHIC PATTERNS SHAPING MICROBIAL COMMUNITIES IN HIGH MOUNTAIN LAKES (PYRENEES, SPAIN)

R. Ortiz-Álvarez[1], J. Catalán[2], L. Camarero[1], X. Triadó-Margarit[1], E. O. Casamayor[1]
[1]Center for Advanced Studies of Blanes CSIC, Continental Ecology, Blanes, Spain
[2]CREAF- Campus UAB- Edifici C- E-08193, CREAF, Cerdanyola del Vallès, Spain

Backgrounds

The lacustrine district in the Pyrenees shows unique characteristics for the study of microbial communities and their distributions in a spatial context. The Pyrenees mountain system is limited by the Cantabrian Sea to the west and the Mediterranean Sea to the east, and its limnological network has evolved since the onset of the last interglacial period. Nowadays, the lacustrine landscape shows very heterogeneous environmental conditions.

Objectives

Understanding the distribution of microbial community types within this region will hint at the general underlying patterns behind community colonization, assembly and diversity.

Methods

We took samples from the lake outlet of ~300 lakes during summer 2011, and applied tag-sequencing of 16S and 18S rRNA genes to disclose bacterial, archaeal and microbial eukaryotes populations identity and community structure.

Conclusions

We fully characterized microbial diversity within this region unveiling a huge degree of novelty and important trophic players within the lake dynamics. Bacteria seem to be assembled by a complex combination of geographic factors and environmental sources of colonization, with a sub-regional structuration. In turn, microbial eukaryotic communities showed strong influence of nutrient content (NO^{3-}, TP and DOC) and especially of water pH, in addition to a geographical signature. Our results indicate that bacterial and microbial eukaryotic communities seem to be distributed and shaped by different chemical and geographic factors.

FEMS7-1245
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PRIMARY SUCCESSION PATTERNS IN MICROBIAL COMMUNITIES ACROSS A WIDE RANGE OF ENVIRONMENTS

R. Ortiz-Álvarez[1], N. Fierer[2], A. de los Ríos[3], E. O. Casamayor[1], A. Barberán[2]
[1]Center for Advanced Studies of Blanes CSIC, Continental Ecology, Blanes, Spain
[2]CIRES- University of Colorado-Boulder, Ecology and Evolutionary Biology, Boulder, USA
[3]National museum of natural sciences- CSIC, Biogeochemistry and microbial ecology, Madrid, Spain

Backgrounds

Ecologists have studied primary succession, the changes that occur in biological communities from initial colonization of an environment, for decades. Most of this work focused on primary succession in plant communities and has established the basis for much of what we currently know about community assembly patterns. Because of their prevalence and importance in ecosystems, there are an increasing number of studies focusing on microbial community dynamics along the primary succession process.

Objectives

Here we aimed to determine whether consistent changes in the diversity, community composition, and functional traits of bacteria over the course of primary succession exist.

Methods

We conducted a meta-analysis of bacterial primary succession samples occurring across a range of distinct habitats including infant gut, plant surfaces, soil chronosequences and water associated communities.

Conclusions

Despite each habitat harbored distinct bacterial communities, we found common patterns, such as the trend of community taxonomic and functional alpha diversity to increase along the succession process. In addition, communities and their functional potential become less variable (lower beta diversity) in the latest successional stages. Finally, consistent shifts in both the rRNA operon copy number and the high efficient phosphate assimilation process (Pst system) were observed, while other functional traits had more ambiguous responses. Overall, these results indicate that successional regularities can be predicted by the underlying general rules of microbial primary succession.

FEMS7-0691
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

A NOVEL PSYCHROTOLERANT METHANOTROPHS OF THE GENUS METHYLOVULUM: ECOPHYSIOLOGY AND INSIGHT INTO GENOMIC FEATURES

I. Oshkin[1], *S. Dedysh*[1]
[1]*Winogradsky Institute of Microbiology, Microbiology, Moscow, Russia*

Backgrounds

Methanotrophs represent a unique group of microorganisms attenuating methane fluxes to the atmosphere. The knowledge on methanotrophs that inhabit low-temperature environments remains limited. This study was focused on two strains of *Methylovulum*-like methanotrophs (Sph1^T and OZ2), which were obtained from two different permanently cold environments and described as a novel species, *Methylovulum psychrotolerans* sp. nov.

Objectives

We aimed to investigate ecophysiology and get insights into genomic features of these bacteria.

Methods

The linear decrease of methane over time in incubations at 5-15 % of methane was used to estimate maximum methane oxidation rates (Vmax). The draft genome sequence of strain Sph1^T was generated using Illumina technology.

Conclusions

Strains grew at 2–32 ^{o}C. Although their optimal growth occurred at 20–25 ^{o}C, they grew very well at lower temperatures, down to 4 ^{o}C. Vmax was in the range of 0.54-1.02×10^{-6} and 0.37-0.39×10^{-6} nmol CH_4 h^{-1} per cell at 10 ^{o}C and 4 ^{o}C, respectively. The total estimated genome size is 5.1 Mb, with an average G+C content of 50.8%. A single rRNA operon, 41 tRNAs, and 4873 predicted protein-coding genes were identified. The genome contains *pmoCAB* operon for pMMO, while sMMO-coding genes are absent. Both *mxa* and *xox* clusters of PQQ-linked dehydrogenases were found. Genes involved in H_4MTP and H_4folate-linked C1 transfer, formate oxidation and dinitrogen fixation were also identified. Complete sets of genes for the function of the RuMP, CBB, TCA, Entner-Doudoroff pathways are present. Thus, analysis of Sph1^T genome confirmed the presence of major genes for methane oxidation and key metabolic pathways.

FEMS7-1063
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIOPROSPECTING OF METHANOGENIC ARCHAEA IN ARCTIC AND ANTARCTIC PERMAFROST

V. Oshurkova[1], V. Shcherbakova[1], E. Rivkina[2]
[1]G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, All-Russian Collection of Microorganisms VKM, Pushchino, Russia
[2]Institute of Physicochemical and Biological Problems in Soil Sciences, laboratory of cryosol, Pushchino, Russia

Backgrounds

Permafrost represents natural deposit of ancient microorganisms which survive at permanently subzero temperatures much longer (over geological epochs) than at any known habitats. Recently some methanogenic archaea were isolated from Arctic permafrost and metagenomic data for six permafrost samples were obtained.

Objectives

The objectives of this work were to investigate the diversity of methanogenic archaea in Arctic permafrost of different origin using metagenomic data, to provide the research of methanogenic population in Antarctic microcosms and to isolate psychroactive methanogenic archaea.

Methods

Based on studied metagenomic data we used different substrates and conditions for stimulation of methanogenesis in numerous enrichments and provided experiment of long-term cultivation of pshychroactive methane-producing microbial community from Arctic permafrost. To monitor the species dynamics in the continuous enrichment thermal gel gradient electrophoresis (TGGE) was used.

Conclusions

The results showed the presence of psychrotolerant representatives of Methanospirillum, Methanosarcina and Methanosphaerula genera in enrichments from some Arctic samples and Antarctic microcosms.

The work was supported by Russian Foundation of Basic Research (grant # 15-04-08612).

FEMS7-2871
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

QUORUM SENSING IN DENTAL PLAQUE: A PARADIGM REVISITED

A. Otero-Casal[1], A. Muras[1], C. Mayer[1], M. Romero[1], N. Mallo[1], J.M. Suarez-Quintanilla[2], P. Otero Casal[2], J.M. Pose-Rodríguez[2]
[1]Faculty of Biology- CIBUS- Universidade de Santiago de Compostela, Department of Microbiology and Parasitology, Santiago de Compostela, Spain
[2]Faculty of Medicine and Dentistry- Universidade de Santiago de Compostela, Department of Surgery and specialized Medical Surgery, Santiago de Compostela, Spain

Backgrounds

Although Quorum sensing (QS) plays a central role in bacterial biofilm formation, its role in dental plaque formation remains unclear. So far the Autoinducer-2 (AI-2) and the peptides produced by oral streptococci are the only QS signals described in pure cultures of oral pathogens, while acyl-Homoserine Lactones (AHLs) have not been reported so far.

Objectives

The detection of AHLs in oral samples to a better understanding of the role of these type of QS molecules in dental plaque formation and in development of bacteria-associated oral pathologies.

Methods

A total of 34 samples of dental plaque from healthy donors and patients presenting different oral pathologies were obtained with a dental explorer, introduced in vials with 500 µL of acidified PBS and extracted with ethyl-acetate. Detection and quantification of AHLs was performed using HPLC-MS. Six extracted teeth were processed and analyzed in the same way.

Conclusions

A wide variety of AHLs was found in both, dental plaque and extracted teeth being C8-HSL the most common signal presents in large amounts in almost all samples. The presence of AHLs in dental plaque and extracted teeth indicate that the QS network in the oral cavity may be much more complex than the accepted paradigm. Although the presence of C8-HSL was not related to the clinical profile of the donors, a different pattern of AHLs was found associated to different pathology profiles. The association of certain AHLs with specific pathologies main ignite new research lines in the field and and opens new treatment possibilities.

FEMS7-3256
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

NEW BIODEGRADATION TESTS FOR CHEMICAL COMPOUNDS AT LOW ENVIRONMENTAL RELEVANT CONCENTRATIONS

B. Ozel Duygan[1], J. van der Meer[1]
[1]Faculty of Biology and Medicine, Department of Fundamental Microbiology, Lausanne, Switzerland

Backgrounds

Assessment of the biodegradation potential of microbial communities is important to judge the environmental fate of polluting substances. Existing biodegradation tests based on oxygen consumption or CO_2 evolution from added substrate are typically carried out at relatively high substrate concentrations (e.g. 20 mgC L^{-1}). However, pollutant concentrations at this level may cause toxicity to microbes and underestimate the potential for biodegradation. Also, since pollutant concentrations in the environment are often much lower, their biodegradation kinetics may be over-estimated from tests at higher concentrations.

Objectives

We aim to develop tests that enable to measure compound biodegradation at concentrations between 0.1-10 mgC L^{-1}.

Methods

The overall methodological concept of our test is based on sensitive measurements of (prokaryotic) community size increase by flow cytometry at the expense of the added compound. We conducted our experiments with aquatic microbial communities from Lake Geneva at initial cell densities of 1E4, 1E5 and 1E6 cells mL^{-1} incubated for up to 7 days. As readily biodegradable substances we tested benzoate, 1-octanol, anthraquinone and phenol, compared to methyl jasmonate, precyclemone B and myrcene.

Conclusions

Community growth up to approximately 1E7 cells mL^{-1} was observed at substrate concentrations of 1-10 mgC L^{-1}. The lowest measurable community size increase was observed for 0.1 mgC L^{-1}. Benzoate, 1-octanol and phenol indeed supported rapid community growth, in contrast to anthraquinone. For benzoate we observed cell aggregation, which complicates community size measurements. Reported conversion coefficients range between 0.02 and 0.2 pgC per cell, with which we obtain up to 60% biomass yield from added compound.

FEMS7-0954
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

USING BOTH LACCASE AND LIGNIN PEROXIDASE ENZYMES FOR PULP BLEACHING

A. Ozer[1], S. Canakci[1], A.O. Beldüz[2]
[1]Karadeniz Technical University, Department of Biology, Trabzon, Turkey
[2]Karadeniz Technical University, Departmant of Biology, Trabzon, Turkey

Backgrounds

Background: Lignin is a heterogeneous polymer that constitutes 30% of woody plant cell walls. Microorganisms that degrade lignin are fungi, actinomycetes and bacteria. Environmental concerns and increasingly stringent emissions standards have led the pulp and paper industry to devise improved treatment technologies. Bleaching with the the enzymes may be very efficient, and can be used under industrial conditions.

Objectives

Objectives: In the light of the current knowledge, the present study focused on the improvement of effictive and synergistic use of laccase and lignin peroxidase biocatalysts on pulp biobleaching. The XOQP TCF sequence was developed as the best pretreatment approach for the biobleaching of pine and eucalyptus kraft pulps .

Methods

Methods: All bleaching experiments were carried out in polyethylene bags by examining different bleaching sequence. The TCF bleaching sequence performed in this study was “XOQP”. X refers to the enzymatic treatment with laccase and lignin peroxidase, O illustrates the oxygen delignification stage, Q represents the chelation treatment, P exemplifies the bleaching with hydrogen peroxide. Washed and oven-dried Kraft pulp was filled into dry polyethylene bags and pretreated with xylanase and lignin peroxidase under optimized experimental conditions. Then, the pulp was subjected to the bleaching sequence for downstream analyses.

Conclusions

Conclusion: The consecutively use of laccase and lignin peroxidase has pulp bleaching effects more higher than the individual enzymes. The usage of the laccase and lignin peroxidase produced from domestic source can reduce external-source dependency, release of harmful chemicals into the environment, processing cost, and improve the biobleaching-based paper quality produced.

FEMS7-0389
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE PHYLOGENETIC DIVERISTY OF CEFTRIAXONE-RESISTANT ISOLATES IN SOIL, DIVERSITY OF THEIR EXTENDED-SPECTRUM B-LACTAMASE (ESBL) GENES, AND THEIR CAPACITY FOR MULTI-DRUG RESISTANCE

E. Pagaling[1], *J. Gatica*[2], *K. Yang*[3], *E. Cytryn*[2], *T. Yan*[3]
[1]*The James Hutton Institute, Ecological Sciences, Aberdeen, United Kingdom*
[2]*Agriculture Research Organisation, Water and Environmental Sciences, Bet-Dagan, Israel*
[3]*The University of Hawaii at Manoa, The Department of Civil and Environmental Engineering, Honolulu, USA*

Backgrounds

The third-generation cephalosporins have been useful in treating infections by Gram negative bacteria as they exhibit wide antimicrobial activity. However, in recent years, increasing levels of resistance have been observed in clinical isolates. Since the soil microbiome is considered to be a natural reservoir of antibiotic resistance genes, soil microbes could potentially pose a risk to public health. However, few studies have reported the presence of extended-spectrum β-lactamase (ESBL) genes in undisturbed soils.

Objectives

1. To determine the phylogenetic diversity of ceftriaxone-resistant soil isolates.
2. To determine the diversity of ESBL genes in ceftriaxone-resistant soil isolates.
3. To determine the extent of multi-drug resistance in ceftriaxone-resistant soil isolates.

Methods

Six undisturbed (non-agricultural or human-impacted) and physiocochemically diverse soils were collected from Hawaii (USA) and Israel. Ceftriaxone-resistant soil isolates were obtained either by irrigation of soil microcosms with clinically relevant concentrations of ceftriaxone, or by creating soil slurries amended with ceftriaxone. The identity of the isolates was determined by sequencing their 16S rRNA genes. To determine their multi-drug resistance, the microdilution method was used to find the minimum inhibitory concentrations (MIC) against 13 other antibiotics as well as ceftriaxone. The isolates were screened for three common ESBL genes by multiplex PCR.

Conclusions

Ceftriaxone-resistance was conferred by a diverse group of microorganisms (within the *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroides*). Higher levels of ceftriaxone-resistance correlated with higher levels of multi-drug resistance. Three distinct ESBL genes were detected in nine genera, suggesting its ubiquitous presence and/or horizontal gene transfer in soil microbiomes.

FEMS7-1188
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ANALYSIS OF 2-(2-HYDROXYLPHENYL)-THIAZOLE-4-CARBALDEHYDE PRODUCTION VIA THE AMBABCDE GENE CLUSTER AND ITS RELATIONSHIP WITH P. AERUGINOSA QUORUM SENSING

A.C. Paiva[1], *F. Matthew*[2], *M. Alexander*[3], *P. Williams*[1]
[1]*The University of Nottingham, School of Life Sciences, Nottingham, United Kingdom*
[2]*Centre for Biomolecular Sciences, School of Life Sciences, Nottingham, United Kingdom*
[3]*The University of Nottingham, School of Pharmacy, Nottingham, United Kingdom*

Backgrounds

Pseudomonas aeruginosa is able to colonize several hosts by producing a variety of virulence factors and secondary metabolites. One of these is the anti-metabolite, L-2-amino-4-methoxy-trans-3-butenoic acid (AMB). AMB biosynthesis is directed by the gene products of the five-gene cluster *ambABCDE*. Recently, these genes have been associated with quorum sensing (QS) in *P. aeruginosa* by directing the biosynthesis of another putative QS molecule, 2-(2-hydroxylphenyl)-thiazole-4-carbaldehyde (IQS). IQS was reported to activate the *rhl* and *pqs* QS systems in an *ambB* mutant, where pyocyanin production was restored under phosphate-limited conditions.

Objectives

Look at IQS production in *amb* mutants compared to the wild type. Analyse the expression of ambA and *ambB* genes under different phosphate conditions. Evaluate how pyocyanin production is affected in these mutants. Understand how this cluster is involved in swarming motility and biofilm development, as *P. aeruginosa* QS is also involved.

Methods

IQS production was measured by LCMS. Transcriptional fusions of *ambA* and *ambB* were created and the expression of these genes was tested in several *amb* and QS mutants, under different phosphate conditions and 200uM IQS. *phzA1* and *phzA2* transcriptional reporter fusions were constructed and the expression and production of pyocyanin was analysed. Lastly, motility and biofilm assays were performed.

Conclusions

Production of IQS and expression of *ambA* and *ambB* was unaltered in *amb* mutants in all conditions tested. Similar occurred in pyocyanin analysis. Moreover, no motility and biofilm defects were noticed. The relationship of the *ambABCDE* genes with IQS production and of the putative IQS signal molecule to QS is therefore unclear.

FEMS7-1305
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

VERTICAL AND SEASONAL DISTRIBUTION OF FUNCTIONAL NITROGEN GENES IN A HIGH-ALTITUDE WARM-MONOMICTIC TROPICAL LAKE

S. Pajares[1], M. Merino-ibarra[1], J. Alcocer[2]
[1]Institute of Marine Sciences and Limnology- UNAM, Ecology and Aquatic Biodiversity, Mexico City, Mexico
[2]FES Iztacala- UNAM, Proyecto de Investigación en Limnología Tropical, Mexico City, Mexico

Backgrounds

The N cycle is highly dependent on microbial processes. Distribution of these communities is one of the most important factors in the variation in N cycling in different aquatic ecosystems. However, little is known about the N transformations and their communities throughout the water column in monomictic tropical lakes.

Objectives

We explored vertical and seasonal patterns of the genetic potential for ammonium oxidation (*amoA*), anammox (*hzo*), DNRA (*nrfA*), and denitrification (*nirS, nirK*) through qPCR to assess the importance of the major N-cycling pathways and their relationships with the main limnological variables in Lake Alchichica (a monomictic tropical lake at Central Mexican Plateau).

Methods

Ten depths were sampled during late stratification (November 2015) and mixing (February 2016) periods, covering the epilimnion, metalimnion (oxycline), and hypolimnion layers.

Conclusions

Thermal and oxygen stratification shaped the distribution of functional N genes in this lake. These genes also varied in relation to nutrient conditions and underwent temporal changes throughout the water column. The *amoA* genes were more abundant during the stratification, mainly at the oxycline layer. Denitrifying genes showed strong variations during this period, with highest gene copy numbers at the oxycline and hypolimnion layers. The *hzo* gene was only detected during stratification at the oxycline and hypolimnium layers. Anoxic conditions were characterized by a relative increase in abundance of the *nrfA* gene, which was positively correlated with NH_4^+ concentration. Our findings highlight the importance of oxygen as one of the main factors influencing the genetic potential for N transformations along the water column in monomictic tropical lakes.

FEMS7-1709
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BACTERIAL COMMUNITY DIVERSITY OF ARTIFICIALLY SUNKEN WOODS IN THE ANTARCTICA SHED LIGHT ON DEEP SEA LARGE ORGANIC FALLS MICROBIAL BIOGEOGRAPHY

C. Palacios[1], M. Zbinden[2], A.G. Glover[3], T.G. Dahlgren[4], M.L. McCormick[5], C.R. Smith[6]
[1]Université de Perpignan Via Domitia- CEFREM-, CNRS- CEFREM- UMR 5110- F-66860, Perpignan, France
[2]Sorbonne Universités- Univ Paris 06- UMR CNRS MNHN 7208 BOREA, Equipe Adaptation aux Milieux Extrêmes, Paris, France
[3]Life Sciences Department- - London- UK, Natural History Museum, London, United Kingdom
[4]Uni Research- Bergen- Norway, University of Gothenburg- Gothenburg- Sweden, Bergen, Norway
[5]Hamilton College, Clinton, NY, USA
[6]University of Hawaii at Manoa, Honolulu, HI, USA

Backgrounds

Sunken wood falls are vegetal deposits resulting from uprooting during storms and carried by rivers to the oceans. During their journey, this debris becomes waterlogged and, when water occupies more volume than air, this woody material sinks to the ocean floor. Wood falls constitute, with kelp and animal remains, especially whale carcasses, large deposits of organic matter in the deep sea, which are thought to have played a major role in the evolution of chemoautotrophically supported communities at the ocean floor.

Objectives

The purpose of our work was to improve our knowledge on how microbial communities living in wood-fall environments evolve and disperse, what has not yet been fully investigated.

Methods

Wood parcels were artificially submerged during one year in the virtually devoid of wood Southern Ocean (Antarctica). We performed electron microscopy and targeted metagenomic analyses to compare microbes mediating wood degradation and microbial diversity in Antarctic sunken wood with those in other ocean waters where wood falls are naturally present.

Conclusions

Photonic and transmission electron microscopy showed that Antarctic microorganisms were able to degrade wood, with patterns of degradation varying according to wood type, as observed in other oceans. Microbial diversities in wood submerged in anaerobic vs. aerobic sediments differed. Nevertheless, microbial communities in Antarctic sunken wood were phylogenetically similar to those from woods immersed for short time periods in other oceans. Our results demonstrate a high dispersal rate and similar evolutionary origin of microorganisms able to colonize organic falls in the deep-sea ocean floor.

FEMS7-2089
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SHIFTING OF BACTERIAL COMMUNITIES IN MEDITERRANEAN WATERCOURSES WITH GEOGRAPHICAL LOCATION, HYDROLOGICAL REGIMES AND ANTHROPOGENIC FORCING, PARTICULARLY DURING MULTICONTAMINATION PHENOMENA

B. Reoyo-prats[1,2], D. Aubert[1,2], O. Verneau[1,2,3], C. Palacios[1,2]
[1]Université de Perpignan Via Domitia, CEFREM UMR5110, F-66860 Perpignan, France
[2]CNRS, CEFREM UMR5110, F-66860 Perpignan, France
[3]University for Environmental Sciences and Management, North-West University, ZA-2520 Potchefstroom, South Africa

Backgrounds

Mediterranean contrasted climate influences river dynamics and therefore the concentration of micropollutants encountered in freshwaters. In a previous study, we have demonstrated that multipollution phenomena occur during heavy rainfall events. Indeed, during dry periods, contaminants get concentrated in rivers watersheds, while at storm events contaminants get remobilized from soils and sediments or come with combined sewer overflows. Thus, Mediterranean rivers are good natural models to study the impact of pollutants mixtures on ecosystems. Moreover, microorganisms are capable of responding very rapidly to variations in the environment, adapting to them, being therefore potential indicators of pollution stress.

Objectives

In this study, we aimed at understanding how bacterial community' diversity changes with variations in pollutants concentrations in river and littoral waters, particularly during recurrent Mediterranean multipollution phenomena.

Methods

A total of 34 samplings were conducted on a typically Mediterranean coastal watercourse, the Têt River, from upstream to downstream, in a complete drought-flood-drought hydrological cycle along 2013-2014. A high frequency monitoring during the flood (13 samplings) at the most impacted station was also performed. Among physic-chemical parameters measurements, up to 350 contaminants have been analyzed including fecal indicators, nutrients, metals, pharmaceuticals and pesticides. A targeted metagenomics analysis was conducted to study bacterial communities.

Conclusions

Multipollution phenomena had a major impact on bacterial communities, among other changes we observed an increment of the opportunistic phylum Proteobacteria during the flood. In addition, beta diversity varies with seasons and stations, but a remarkable change was observed at the multipollution peak during heavy rain periods.

FEMS7-2092
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHANGES IN ARCHAEAL COMMUNITY STRUCTURE DIVERSITY ALONG WITH VARIATIONS IN POLLUTANTS CONCENTRATIONS IN A TYPICAL MEDITERRANEAN COASTAL RIVER

B. Reoyo-prats[1,2], J. Ezagouri-larra[1,2], C. Palacios[1,2]
[1]Université de Perpignan Via Domitia, CEFREM UMR5110, F-66860 Perpignan, France
[2]CNRS, CEFREM UMR5110, F-66860 Perpignan, France

Backgrounds

Assessing the environmental impact of anthropogenic contaminants in aquatic environments represents a fundamental issue. Micropollutants concentrations in watercourses depend on numerous factors such as land use and rainfall events. Particularly relevant are the contrasting hydrodynamic regimes of droughts and floods at Mediterranean coastal rivers. We have shown that typical heavy rainfalls in these regions are at the origin of multipollution phenomena. In this context, microorganisms attached to suspended solids need a better consideration as markers of aquatic ecosystems reactivity to pollutants because these biofilms are the first to interact with contaminants, degrading or transforming them.

Objectives

In this study, we focused on the impact of contaminants changes on the structural diversity of archaeal biofilms associated to the Têt River, a major coastal watercourse in the South-East of France.

Methods

Water quality measurements, including the analyses of more than 300 micropollutant molecules, were performed from upstream to foreshore waters for a total of 34 samples, in a complete drought-flood-drought hydrological cycle along 2013-2014. A high frequency sampling during the flood (13 samples) at the most impacted station of the Têt River was also carried out. The diversity of archaeal communities attached to suspended matter in these samples was studied using targeted metagenomics analysis on the 16S rRNA gene.

Conclusions

Archaeal communities were composed of two main phyla, Thaumarchaeota and Euryarchaeota. Variations in phylogenetic composition within these phyla were observed among drought versus heavy rainfalls samples. Moreover, an archaeal taxonomic diversification could be directly linked to changes in pollutants levels during heavy rainfalls.

FEMS7-2485
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECT OF FOUR DIFFERENT WASTEWATER TREATMENTS IN THE REDUCTION OF ANTIBIOTIC RESISTANCE GENES

R. Pallarés-Vega[1,2], L. Hernández-Leal[3], H. Schmitt[4], D.G. Weissbrodt[2], M.C. van Loosdrecht[2]
[1]Wetsus, Microbiology, Leeuwarden, The Netherlands
[2]Delft University of Technology, Biotechnology, Delft, The Netherlands
[3]Wetsus, Source Separation Sanitation., Leeuwarden, The Netherlands
[4]University of Utrecht., Veterinary Medicine, Utrecht, The Netherlands

Backgrounds

Antibiotic Resistance (AR) is considered a public health threat. Despite it is known to be ubiquitous in nature, there is a great concern of increasing the environmental resistome by spreading anthropogenic Antibiotic Resistance Bacteria (ARB) and Genes (ARGs) into the environment. Wastewater treatment plants (WWTPs) treat fecal residues and are suspected as one of putative hotspots of breed and spread of AR from human sources into aquatic ecosystems. Special attention is brought on their biological process (BP) in which genetic exchange might occur among incoming and indigenous bacterial community.

Objectives

The kind of sludge, the retention time and/or the addition of chemicals might make a difference in the AR removal rate. In order to assess the AR removal efficiency of different treatments, we studied three selected WWTPs harbouring four diverse BP: Two Conventional Activated Sludge (different Phosphorus removal), and a third plant harbouring a paralel treatment (i) AB system and (ii) Nereda.

Methods

Water line samples (24h flow proportional composite) and sludge line samples (grab samples) were collected monthly from all the stages of each WWTPs. Quantification of a panel of 9 relevant ARGs namely *ctxM*, *tetM*, *qnrS*, *sul1*, *sul2*, *mecA*, *ermB*, *vanA and intl1* was conducted by means of qPCR.

Conclusions

Preliminary results showed a high prevalence (10^5-10^6 gene copies/ml) of all the genes in the raw influent except for *mecA* and *vanA*. After treatment, 3logs of reduction were achieved, although some genes as *sul1* remained 10 fold more prevalent. Further data analyses are needed to conclude the differences between the treatments.

FEMS7-1848
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ORGANIC ACID PRODUCTION BY FUNGI: COMPARISON ON VARIOUS MEDIA AND EFFECT OF THE INTERACTION WITH BACTERIA

F. Palmieri[1], S. Bindschedler[1], P. Junier[1]
[1]Institute of Biology, Laboratory of Microbiology, Neuchâtel, Switzerland

Backgrounds

In fungi, low molecular weight organic acids (LMWOA) production can have important roles in processes as diverse as pathogenesis, competition, mineral weathering or lignocellulose degradation. More recently, LMWOA have been identified as being involved in the interaction between fungi and bacteria. This is particularly true in the case of oxalic acid, especially in soils. Several factors can influence LMWOA production. One of those is the availability of divalent cations. They trigger LMWOA production either as enzymatic co-factors or as a detoxification mechanism.

Objectives

The aim of this study is to evaluate the production of LMWOA in fungi on different media, in the presence of divalent cations (i.e. Mn^{2+} or Ca^{2+}), as well as bacteria. In this way the effect of cations and interactive bacteria in LMWOA production can be assessed.

Methods

LMWOA production in selected fungi will be confirmed by using various culture media and subsequent acid detection and quantification. Co-cultures of fungi and soil bacteria that interact with fungi will then be performed. LMWOA production will also be assessed chemically and the production of mineral complexes in the presence of divalent cations will be measured with optical microscopy or SEM.

Conclusions

Half of the fungal strains initially screened produced large amounts of organic acids in all media tested. With this study, we can gain new insights concerning the factors influencing LMWOA metabolism in fungi and the effect of those metabolites in the interaction with bacteria.

FEMS7-3228
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECT OF TRICHODERMA BREVICOMPACTUM AND DROUGHT ON STRESS RELATED GENES OF TOMATO PLANTS

D. Panković[1], G. Racić[1], I. Vukelić[1], N. Ćurčić[1], L. Prokić[2]
[1]Educons University, Faculty of ecological agriculture, Sremska Kamenica, Serbia
[2]University of Belgrade, Faculty of agriculture, Zemun, Serbia

Backgrounds

Trichoderma brevicompactum belongs to the *Brevicompactum* clade and it is known for the production of trichothecene-type mycotoxins,phytotoxins and inhibitors of protein synthesis in mammalian cells. Investigations on the effect of *T. brevicompactum* on plants are limited, as compared to those of the other species such as *T. viride* and *T. harzianum*. Moreover, the cross-talk between signaling pathways involved in *Trichoderma* mediated plant response to drought, as the most frequent abiotic stress, is still not clear.

Objectives

The expression of several stress related genes: markers of salicylic acid (SA) signaling pathway, jasmonic acid (JA) pathway and abscisic acid (ABA) related,in tomato root and leaves was examined in response to *T.brevicompactum* treatment only, or in combination with drought stress.

Methods

Tomato plants (Lycopersicon esculentum Mill. cv. Ailsa Craig) were used in this study. *T. brevicompactum* SZMC 22661 suspension was added to the root zone. Effect of *Trichoderma* treatment, on both drought stressed and control plants on the expression of stress related genes in tomato was examined by quantitative reverse transcription real-time polymerase reaction (qRT-PCR).

Conclusions

Analysis of gene expression in tomato leaves and roots, in response to both investigated stress factors- drought and the application of *T. brevicompactum* SZMC 22661, indicates that different signaling pathways are activated in the leaves and roots. Results indicated that SA, JA and ABA signaling pathways are involved in reaction to both introduced stresses. The mutual effect of investigated stresses leads to the modification of gene expression in comparison to the reaction to the single stress.

FEMS7-1557
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SELECTIVE NEXT GENERATION SEQUENCING-BASED AUDITING OF PROBLEMATIC MICROBIAL GROUPS IN WATER

A. Paparini[1], E. Lee[1], M. Khurana[1], A. Whiteley[2], P. Monis[3], A. Bath[4], C. Gordon[4], U. Ryan[1]
[1]Murdoch University, Vector- and Water-
Borne Pathogen Research Group School of Veterinary and Life Sciences, Murdoch WA, Australia
[2]The University of Western Australia, School of Earth and Environment, Crawley WA, Australia
[3]South Australian Water Corporation, Australian Water Quality Centre, Adelaide SA, Australia
[4]Water Corporation of WA, Drinking Water Quality Branch, Leederville WA, Australia

Backgrounds

Next generation sequencing (NGS) has rapidly become an invaluable tool for the detection, identification and relative quantification of environmental microorganisms. Universal PCR primers targeting the 16rRNA gene are typically used to perform high-throughput biodiversity assessments. Ideally, this approach is unselective and, by amplifying all taxa with equal efficiency, it aims at collecting genetic– and quantitative–information from all species present in the microbial community.

Objectives

Here, we present two new NGS-compatible primer sets, which are designed to specifically target only potentially problematic microbial groups, during water quality studies.

Methods

The primers were designed from an alignment of 6,513 cyanobacteria 16S rDNA sequences, and NGS was performed by Ion Torrent PGM. Bacterial cultures and heterogeneous types of environmental waters were tested.

Conclusions

Compared to universal primers, in silico and experimental analyses demonstrated that the new primers showed increased specificity for Cyanobacteria and Proteobacteria, allowing increased sensitivity for the detection, identification and relative quantification of toxic bloom-forming microalgae, microbial water quality bioindicators and common pathogens. Significantly, Cyanobacterial and Proteobacterial sequences accounted for ~95% of all sequences obtained within NGS runs (when compared to ~50% with standard universal NGS primers), providing higher sensitivity and greater phylogenetic resolution of key microbial groups affecting water quality. The increased selectivity of the new primers allow parallel sequencing of more samples through reduced sequence retrieval levels required to detect target groups, potentially reducing NGS costs by 50% but still guaranteeing optimal coverage and species discrimination.

FEMS7-1281
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COPPER NANOPARTICLES IN SOIL: EFFECT ON MICROBIAL COMMUNITIES AND ATRAZINE DEGRADATION

J. Parada[1], O. Rubilar[2], N. Millahueque[1], M. Martinez[3], G. Tortella[4]
[1]Universidad de La Frontera, Departamento de Ingenieria Quimica, Temuco, Chile
[2]Universidad de La Frontera, Departamento de Ingeniería Quimica, Temuco, Chile
[3]Universidad de Concepción, Departamento de Microbiología, Concepción, Chile
[4]Universidad de La Frontera, Departamento de Ingenieria Química, Temuco, Chile

Backgrounds

Copper nanoparticles (Nano-Cu) have been included on many products due to the antimicrobial effect of copper. However, the potential impact of Nano-Cu on the environment and the possible co-existence with pesticides in soil has not been studied.

Objectives

Therefore, the aim of this study was to evaluate the effect of Nano-Cu combined with atrazine (ATZ) on the soil ability to degrade this pesticide and the consequent impact on soil microbial communities.

Methods

The main activities that were carried out were to pollute soil (Andisol) with increasing concentrations of commercial Nano-Cu (500 and 1500 mg kg^{-1}) and ATZ at field dose (3 mg kg^{-1}). ATZ residual was measured by HPLC to evaluate the ATZ degradation capacity at different time intervals (0, 10, 20 and 30 days). Changes on the structural diversity of soil microbial communities were observed by PCR-Denaturing Gradient Gel Electrophoresis (DGGE), with universal primers (16S and 18S rDNA for bacteria and fungae, respectively). The abiotic impact in soil was evaluated through adsorption-desorption experiments. Copper sulfate ($CuSO_4$) was used as control.

Conclusions

The results showed that Nano-Cu (1500 mg kg^{-1}) affected significantly the ability to degrade atrazine (10-fold reduced after 30 days), and this effect was major than $CuSO_4$. It was also observed a major adsorption of ATZ as the Nano-Cu concentration increased. DGGE patterns demonstrated that microbial community structure remained relatively stable in time when compared to controls. In conclusion, our results demonstrated that Nano-Cu in soil have a negative impact on atrazine degradation capacity potentially related to physical-chemical processes in soil.

FEMS7-2158
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ENDOPHYTIC HERBASPIRILLUM SP. AND BURKHOLDERIA TROPICA ISOLATES PROMOTE ALUMINIUM TOLERANCE IN SUGARCANE SEDDLINGS

E. Labanca[1], A.P.D. Silveira[1], E. Kuramae[2], S.A.L. Andrade[3], C.L. Mazzolani[1]
[1]Agronomic Institute, Center of Soil and Environmental Resources, Campinas, Brazil
[2]Netherlands Institute of Ecology NIOO-KNAW, Department of Microbial Ecology, Wageningen, The Netherlands
[3]University of Campinas, Institute of Biology, Campinas, Brazil

Backgrounds

Plant-growth promoting bacteria (PGPB) are known for their ability to increase plant biomass and to improve plant fitness, especially under adverse conditions. Aluminium (Al) induces several disorders in plants such as plasmatic membrane changes, nucleic acids damage, reative oxigen species production and negative impact on nutrient uptake and transport. On the other hand, plants reduce these negative effects by increasing the activity of antioxidant enzymes and the content of proline and phenolics compounds.

Objectives

Two bacteria species (*Burkholderia tropica* and *Herbaspirillum* sp.), isolated from sugarcane roots with PGPB properties were tested in order to evaluate their potencial to enhance the crop capability to cope with Al stress.

Methods

In this study, the two bacteria were inoculated in sugarcane plantlets grown in a soil with high Al content for 32 days.The bacteria-plant interaction resulted in: (1) Increase of the activities of superoxide dismutase, ascorbate peroxidase and peroxidase in roots; (2) High concentration of Al in the shoots; (3) Increment of proline and aromatic amino acids in the leaves; (4) Increase of plant biomass and water content.

Conclusions

Al transport to the shoots, associated with the higher activity of the antioxidant enzymes in the roots, probably alleviated the Al damage to the roots. The increment of aromatic amino acids points to an enhancement in the shikimate pathway, route related to the biosynthesis of several defense compounds. The results suggest that these bacteria species induced sugarcane acclimatation to the Al stress.

FEMS7-0212
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DETECTION AND DIVERSITY OF THE NITRITE OXIDOREDUCTASE ALPHA SUBUNIT (NXRA) GENE OF NITROSPINA IN MARINE SEDIMENTS

S.J. Park[1], K. Hyeon-Woo[1], F. Hirotsugu[2]
[1]Jeju National University, Department of Biology, Jeju, Republic of Korea
[2]Waseda University, Department of Life Science and Medical Bioscience, Tokyo, Japan

Backgrounds

Nitrite-oxidizing bacteria (NOB) are chemolithoautotrophs that catalyze the oxidation of nitrite to nitrate, which is the second step of aerobic nitrification. In marine ecosystems, *Nitrospina* is assumed to be a major contributor to nitrification. To date, two strains of *Nitrospina* have been isolated from marine environments.

Objectives

Despite their ecological relevance, their ecophysiology and environmental distribution are understudied owing to fastidious cultivation techniques and the lack of a sufficient functional gene marker. To estimate the abundance, diversity, and distribution of *Nitrospina* in various marine sediments, we used *nxrA*, which encodes the alpha subunit of nitrite oxidoreductase, as a functional and phylogenetic marker.

Methods

In this study, we explored and compared *Nitrospina* diversity in six marine sediments, including samples from both polar regions, using pyrosequencing.

Conclusions

We observed that *Nitrospina* diversity in polar sediments was significantly lower than that of non-polar samples. Moreover, *nxrA*-like sequences revealed an unexpected diversity of *Nitrospina*, with approximately 41,000 different sequences based on a 95% similarity cut-off from six marine sediments. We detected *nxrA* gene copy numbers of up to 3.57×10^4 per gram of marine sediment sample. The results of this study provide insight into the distribution and diversity of *Nitrospina*, which is fundamentally important for understanding their contribution to the nitrogen cycle in marine sediments.

FEMS7-0521
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DUAL-ACTION OF AEROBIC NON-UREOLYTIC AND ANAEROBIC DENITRIFYING LYSINIBACILLUS SP. YS11 FOR CALCIUM CARBONATE PRECIPITATION

Y.S. Lee[1], H.J. Kim[1], W. Park[1]
[1]Korea University, Environmental Sciecne and Ecological Engineering, Seoul, Republic of Korea

Backgrounds

Microbially induced calcium carbonate precipitation (CCP) occurs through different metabolic pathways. Although CCP through ureolysis has been widely considered in environmental engineering fields, urea utilization might cause environmental problems due to production of ammonia and nitrate

Objectives

In this study, several non-ureolytic CCP bacteria that induced alkali pH were isolated from rhizosphere of *Miscanthus sacchariflorus* near artificial stream and their non-ureolytic mechanisms for CCP was investigated

Methods

CCP using phase-contrast microscopy and ion-selective electrode was observed. Only *Lysinibacillus* sp. YS11 has shown dual-action for CCP: aerobic non-ureolytic and anaerobic denitrification. Energy dispersive X-ray spectrometry mapping confirmed the presence of calcium carbonate. Field emission scanning electron microscopy analysis indicated morphologically distinct minerals formation under those conditions.

Conclusions

Resistance in alkali pH and high salt concentrations and spore formation of strain YS11 advocate potential applicability in self-healing concrete. Successive monitoring of growth, pH, and calcium ion using CFU counting, pH meter and ion-selective electrode suggested that strain YS11 was able to induce alkaline condition up to pH 9 and utilize 96% of free Ca^{2+}. It is first to report dual-action of bacteria for CCP under aerobic and anaerobic conditions.

FEMS7-0748
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DEHALOGENIMONAS-DRIVEN 1,2-DICHLOROPROPANE-DEGRADING CONSORTIUM: ESTABLISHMENT AND COMMUNITY ASSESSMENT

E. Parlade[1], S.H. Mortan[2], M. Llirós[1], L. Martín-González[2], G. Caminal[3], E. Marco-Urrea[2], N. Gaju[1], M. Martínez-Alonso[1]
[1]Universitat Autònoma de Barcelona, Department of Genetics and Microbiology, Bellaterra, Spain
[2]Universitat Autònoma de Barcelona,
Department of Chemical Biological and Environmental Engineering, Bellaterra, Spain
[3]Institut de Química Avançada de Catalunya IQAC-CSIC, -, Barcelona, Spain

Backgrounds

Organochlorides are among the main pollutants in aquifers, especially those close to historically contaminated sites, representing a health risk for consumers depending on groundwater supply.

Objectives

The aim of this study is to describe the consolidation process of an anaerobic bacterial consortium with great bioremediation potential due to its metabolic capabilities to transform 1,2-dichloropropane (1,2-DCP) to propene.

Methods

Bacterial community composition and 1,2-DCP degradation rates were determined at three different stages during consortia development using three different molecular techniques (i.e., DGGE, clone libraries, and Illumina sequencing) and gas chromatography, respectively. The abundance of *Dehalogenimonas,* the genus responsible of DCP degradation, was assessed through real-time qPCR along the 19 months of activity.

Conclusions

The consortium in the final stage was clearly dominated by *Dehalogenimonas* and *Azonexus* genera, while other accompanying microorganisms such as *Desulfovibrio*, *Geobacter* or *Sphaerochaeta* exhibited modest concentrations. Enrichment of *Dehalogenimonas* genus was further demonstrated by specific 16S rRNA gene qPCR and ultimately lead to overall higher degradation rates. Our results confirmed a specialized community development in the consortia with potential to maintain the dechlorinating activity in the natural environment. Most of the abundant genera detected are known to supply either essential factors or growth enhancers useful to the organohalide-respiring bacteria. In parallel, comparison among molecular techniques revealed that dominant genera were detected in all tested methodologies and low-abundant sequences remained undervalued in DGGE and clone library approaches.

FEMS7-2031
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DEVELOPMENT AND VALIDATION OF A NEW PHYLOGENETIC DNA-MICROARRAY SPECIFIC FOR THE ORAL MICROBIOTA

C. Parolin[1], B. Giordani[1], R.A. Ñahui Palomino[1], E. Biagi[1], M. Severgnini[2], C. Consolandi[2], G. Caredda[2], S. Storelli[3], L. Strohmenger[3], B. Vitali[1]
[1]University of Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy
[2]National Research Council, Institute of Biomedical Technologies, Segrate- Milan, Italy
[3]University of Milan,
Department of Biomedical Surgical and Dental Sciences- ASST Santi Paolo e Carlo, Milan, Italy

Backgrounds

The oral cavity contains hundreds of different microbial species, and their quali-quantitative characterization is crucial for an exhaustive comprehension of the oral ecology. The study of the human oral microbiota and the modifications of the microbial composition that occur during the most common odontoiatric pathologies, such as dental caries, periodontitis and perimplantitis, is of great interest.

Objectives

The objective of the present study was to develop a phylogenetic DNA-microarray, named OralArray, to quickly and reliably characterize the most representative bacterial groups that colonize different sites of the oral cavity in healthy and pathological conditions.

Methods

The OralArray was based on the Ligation Detection Reaction technology associated to Universal Arrays, and includes 22 probe sets targeted to bacteria belonging to the phyla *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Fusobacteria* and *Spirochaete.* The OralArray was tested and validated on different types of oral samples (saliva, lingual plaque, supragingival plaque and healing cap), collected from 10 healthy subjects.

Conclusions

The phylogenetic microarray is characterized by high specificity, high sensitivity (down to 1 ng of PCR product) and a reproducibility of 97.7%. The OralArray was able to detect the microbial signature of different types of oral samples. Our results established the presence of an oral microbial signature specific for each subject, rather than for sample type. Moreover, the molecular tool was employed to evaluate the efficacy of a disinfectant treatment on the healing caps before their usage. The OralArray is thus suitable to study the microbiota associated with various oral sites and to monitor changes arising from therapeutic treatments.

FEMS7-2102
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE VAGINAL MICROBIOME OF HEALTHY, BACTERIAL VAGINOSIS AND CHLAMYDIA TRACHOMATIS-INFECTED WOMEN

C. Parolin[1], C. Foschi[2], L. Laghi[3], B. Giordani[1], V. Gaspari[4], A. D'Antuono[4], R. Cevenini[2], A. Marangoni[2], B. Vitali[1]
[1]University of Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy
[2]University of Bologna,
Department of Experimental Diagnostic and Specialty Medicine- section of Microbiology, Bologna, Italy
[3]University of Bologna, Department of Agro-Food Science and Technology- Centre of Foodomics, Cesena, Italy
[4]University of Bologna,
Department of Experimental Diagnostic and Specialty Medicine- section of Dermatology, Bologna, Italy

Backgrounds

The vaginal microbiota of healthy women is generally dominated by *Lactobacillus* spp., which are known to protect the female genital tract from microbial dysbiosis and pathogen overgrowth. Alterations of the vaginal microbiota composition are found in bacterial vaginosis (BV). Lactobacilli have also been hypothesized to protect women from sexually transmitted diseases, including *Chlamydia trachomatis* (CT) infection, which represents the most common bacterial sexually transmitted infection worldwide. Both BV and CT infection can lead to severe sequelae and complications, including preterm labor and delivery, and tubal infertility.

Objectives

The aim of this study is to analyse the composition of the endogenous microbiota and the metabolic profiles of the vaginal niche in three different conditions: healthy, BV and CT infection.

Methods

Vaginal swabs were obtained from 66 women, belonging to three groups (healthy, BV and CT-infected women). The microbial composition of the samples was determined by using a microarray-based tool (VaginArray) targeting the most representative bacterial groups of the vaginal ecosystem, together with a quantitative real-time PCR for *Gardnerella vaginalis.* The metabolic profiles of the vaginal specimens were assessed by ^{1}H-NMR.

Conclusions

From our results the microbial signature of BV-affected women is clearly different from that found in healthy subjects, CT-infected women are characterized by a microbiota similar to the healthy condition. The metabolomics approach evidenced that BV-samples are characterized by significant variations in organic acids, aminoacids, short chain fatty acids concentrations. CT-infected women metabolome resembles that of healthy subjects, nevertheless significant variations in biogenic amines content were underlined.

FEMS7-1379
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COPPER NANOPARTICLES INTERFERE THE TRANSFERENCE, BY CONJUGATION, OF A PLASMID BETWEEN CUPRIAVIDUS PINATUBONENSIS JPM134 AND PSEUDOMONAS PUTIDA KT2440.

M. Martínez[1], *G. Tortella*[2]
[1]*Universidad de Concepción, Microbiología, Concepción, Chile*
[2]*Universidad de la Frontera, Ingeniería Química, Temuco, Chile*

Backgrounds

In the last decade, engineered nanoparticles (NPs) are being increasingly used in diverse products. Antimicrobial properties of metallic NPs, such as copper (CuNps), have been reported in numerous studies. The toxicity mechanism is generated by the contact of NPs with microbial membranes, causing its disruption, oxidation of its proteins or interruption of energy transduction. Some soil bacteria possess plasmids carrying genes that allow the biodegradation of toxic compounds and they are frequently transferred by conjugation to other bacteria present in soil. In this context, the presence of CuNps could negatively affect the cell-cell contact necessary for this process.

Objectives

The aim of this study was to determine the effect of CuNps on the transference by conjugation of plasmid pJP4, containing genes necessary to degrade 2,4-dichlorophenoxyacetic acid, from *Cupriavidus pinatubonensis* JPM134 to *Pseudomonas putida* KT2440.

Methods

A 9:1 donor: receptor mixture was incubated during 2 h in R2A broth at 30ºC in the presence of CuNps (40-60nm), in a series of subinhibitory concentrations (0, 10, 20, 50, 100, 200 µg ml^{-1}). Transconjugants were selected on R2A agar containing chloramphenicol (40 µg ml^{-1}) and gentamicin (15 µg ml^{-1}). The transference of pJP4 was confirmed by PCR of the *tfd*B gene.

Conclusions

Results indicated that the presence of >50 µg ml^{-1} CuNps caused a significant reduction of conjugation frequency (*c.a*. 90%), from $4{,}4x10^{-6}$ to $5x10^{-5}$ UFC ml^{-1}. Therefore, CuNps may interfere with the transference of plasmids containing genes allowing the biorremediación of toxic compounds, reducing the biodegradative potential of microbial soil communities.

FEMS7-0334
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE ECOLOGICAL DYNAMICS OF BACTERIAL PRODUCTION OF CADAVERIC SEMIOCHEMICAL MOLECULES DURING MAMMALIAN CARCASS DECOMPOSITION IN SITU

J. Pascual[1], C. von Hoermann[2], A.M. Rottler-Hoermann[2], O. Nevo[2], A. Geppert[1], J. Sikorski[1], K.J. Huber[1], S. Steiger[2], M. Ayasse[2], J. Overmann[1]
[1]DSMZ, Microbial Ecology and Diversity Research, Braunschweig, Germany
[2]Institute of Evolutionary Ecology and Conservation Genomics- University of Ulm, Evolutionary Ecology and Conservation Genomics, Ulm, Germany

Backgrounds

The temporal dynamics of taxonomic and functional shifts of epinecrotic bacteria – major drivers of the decomposition of dead mammalian tissue – are only weakly explored.

Objectives

The main goal of the present study is to identify the systematic temporal changes in bacterial composition and function during the first stages of carcass decomposition. The characteristics of this process may drive other key players of carcass decomposition such as scavenger insects.

Methods

Carcass decomposition was monitored using still-born piglets incubated in different forest ecosystems for 96 hours. High-throughput Illumina 16S rRNA sequencing (bacterial community composition) was combined with gas chromatography-mass spectrometry of individual cadaveric volatiles produced by microbial degradation. The data were analyzed using mono- and multivariate statistics.

Conclusions

The taxonomic composition of epinecrotic bacteria changes over time while simultaneously alpha-diversity increases and beta-diversity decreases, suggesting deterministic and not stochastic processes. The cadaveric volatile compound composition changes similarly, suggesting a significant impact of the bacterial community. The synthesis of acetic acid, indole and phenol could be linked to the activity of *Enterobacteriaceae*, *Tissierellaceae* and *Xanthomonadaceae*, respectively. Based on identified volatiles (acetic acid, indole and phenol) which attract insects, we additionally hypothesize that temporal recruitment of macroscopic scavengers is also driven by bacterial function. This is the first report that attributes the *in situ* synthesis of cadaveric volatile organic compounds to particular types of epinecrotic microorganisms, and thus provides the basis for future further chemical ecology and forensic studies.

FEMS7-2482
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RHIZOSPHERE MICROBIAL COMMUNITY ASSEMBLY IN RESPONSE TO DIFFERENT NUTRIENT INPUTS

F. Paula[1], E. Tatti[1], C. Thorn[1], F. Abram[1], J. Wilson[2], V. O'Flaherty[1]
[1]National University of Ireland- Galway, Microbiology, Galway, Ireland
[2]Monaghan Mushrooms, Research and Development department, Tyholland, Ireland

Backgrounds

The rhizosphere is a highly complex environment in which an array of ecological interactions between plant and microbes takes place. It represents a hotspot supporting microbial communities whose composition may differ from adjacent bulk soil, where nutrients are often limited. Despite the indications that changes in these communities may have implications in plant productivity, it is still unclear how their composition, diversity and turnover are affected by nutrient inputs.

Objectives

We aimed to evaluate how organic *versus* inorganic amendments impacted prokaryotic and fungal community assembly in *Lolium multiflorum* rhizosphere.

Methods

In a soil trial, stabilised spent mushroom compost was applied as sole source of NPK and microbial communities compared to soil treated with mineral fertilizer. Rhizosphere and bulk soil fractions were collected for 14 weeks and the composition of the community was assessed by barcode sequencing targeting the regions flanked by the primers 515F-806R (prokaryote) and ITS3F-ITS4R (fungi). Metaproteomics analysis was carried out to investigate changes in functional diversity.

Conclusions

Microbial community composition was significantly different among treatments, as revealed by taxonomic and functional data. Clear community turnover was observed with time. Increasing diversity was observed in both prokaryotic and fungal communities at later stages of plant development. Moreover, high microbial diversity, but not richness, was associated with high plant productivity. Finally, distinctions between bulk and root associated communities were less evident under organic treatment, in comparison to mineral. This suggests that the large inputs of organic matter may minimize differences in nutrient availability between the two soil fractions.

FEMS7-0613
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IDENTIFYING THE SPREAD OF RESISTANT E. COLI ISOLATES IN HOSPITAL AND COMMUNITY SEWAGE AND THEIR RECEIVING SEWAGE TREATMENT PLANT

E. Paulshus[1], K. O'Sullivan[1], I. Kuhn[2], P. Colque-Navarro[2], R. Möllby[2], H. Sørum[1]
[1]Norwegian University of Life Sciences, Department of Food Safety and Infection Biology, Oslo, Norway
[2]Karolinska Institutet, Department of Microbiology- Tumor and Cell Biology, Stockholm, Sweden

Backgrounds

Antibiotic resistance (AR) is a continuingly increasing threat to global health. Certain antibiotic resistant bacteria (ARBs) have previously been found in hospital and urban sewage before the same clones appear among clinical isolates, probably reflecting a low prevalence in the population. Thus, analysis of bacteria in sewage, especially from hospitals, might facilitate an indicator and early warning system for ARBs.

Objectives

We try to identify major sewage outlets with regard to ARBs before high-risk outlets reach the main sewage system. The results will be used to establish proper handling of drug resistant bacteria at certain hot-spots in the sewage system. This may be cost-effective compared to handling all sewage in the major sewage plant with respect to reducing the level of drug resistant bacteria.

Methods

Samples were collected monthly for 18 months from hospital (HS) and community sewage (CS), and from a sewage treatment plant (STP). *E. coli* were selectively cultivated (CHROMagar Orientation, 44°C) and their phenotypic relatedness and AR patterns analyzed using the *PhenePlate-AREB system*.

Conclusions

A total of 7221 *E. coli* isolates have been analyzed so far. Elevated resistance in HS compared to CS was observed towards several antibiotics. HS showed the highest overall resistance to the tested antibiotics (Multiple Antibiotic Resistance (MAR) index = 0.16), followed by CS (MAR = 0.15), whereas the resistance level in STP was lower than in HS and CS for all antibiotics tested (MAR=0.09). This indicates a dilution effect of the ARBs before they reach the STP.

FEMS7-1549
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PATHOGENIC E. COLI: NEW INSIGHTS INTO THE REGULATION AND INHIBITION OF BACTERIAL AGGREGATION/BIOFILM FORMATION

J. Paxman[1], A. Lo[2], S. Panjikar[3], T. Wang[1], C. Darmanin[4], M. Schembri[2], B. Heras[1]
[1]La Trobe Institute for Molecular Science, Biochemistry and Genetics, Melbourne, Australia
[2]The University of Queensland, Australian Infectious Diseases Research Centre, Brisbane, Australia
[3]Australian Synchrotron, Macromolecular Crystallography, Clayton, Australia
[4]La Trobe University, ARC Centre of Excellence in Advanced Molecular Imaging, Melbourne, Australia

Backgrounds

The self-associating autotransporters (SAATs) are a group of glycosylated proteins from widespread *E. coli* pathogens that are transported to the cell surface by the type V secretion pathway. These virulence factors have central roles in bacterial aggregation and biofilm formation, which are important attributes for colonisation and persistence, which is extremely relevant to the areas of bacterial pathogenesis, nonsocomial infections and food sanitation. We recently elucidated the mechanism by which the SAAT Antigen43 from uropathogenic *E. coli* (UPEC) promotes bacterial aggregation/biofilm formation, by means of self-association between neighbouring cells[1].

Objectives

We sought to determine if all SAATs shared a common mechanism for facilitating bacterial aggregation/biofilm formation, if this function was regulated and if it could be inhibited.

Methods

The SAAT TibA from enterotoxigenic *E. coli* (ETEC) was known to be glycosylated by the TibC glycosyltransferase. We determined the crystal structures of the glycosylated and unglycosylated forms of TibA and used this to inform further biophysical and phenotypic studies. We found that TibA self-associates similarly to that of Antigen43 to facilitate bacterial aggregation/biofilm formation, but with a more extensive interface. Glycosylation by TibC was found to physically block TibA self-association to reduce bacterial aggregation/biofilm formation. We are now developing specific inhibitors for SAAT mediated aggregation/biofilm formation and have determined the first Antigen43-inhibitor crystal structure.

Conclusions

In summary, we have used our 3 new autotransporter crystal structures to inform the mechanism of SAAT mediated bacterial aggregation/biofilm formation, to elucidate how glycosylation regulates SAAT function and to develop a strategy to block these virulence processes.

FEMS7-0373
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PHYTOPLANKTON IDENTIFICATION WITH CHEMOTAXONOMIC BIOMARKERS: IN COMBINATION THEY MAY DO BETTER

E. Peltomaa[1], M. Tossavainen[1], S. Taipale[2]
[1]University of Helsinki, Environmental Sciences, Lahti, Finland
[2]University of Jyväskylä, Biological and Environmental Science, Jyväskylä, Finland

Backgrounds

The composition of phytoplankton community is important when defining aquatic production, since phytoplankton synthesize many taxon-specific biomolecules, which consumers cannot synthesize *de novo*. Chemotaxonomic biomarkers are needed for monitoring phytoplankton communities and the nutritional quality of seston for upper trophic levels.

Objectives

Whereas the fatty acid and pigment profiles of phytoplankton are known to be mainly taxon-specific, sterols have not been successfully established as chemotaxonomic biomarkers. Neither have all these three been used concomitantly for the characterization of phytoplankton communities in nature. Here we studied the suitability of the combination of fatty acids, sterols and carotenoids as chemotaxonomic markers using multivariate statistics.

Methods

Our analysis included 40 phytoplankton strains from 10 classes isolated from freshwater lakes. The fatty acid and sterol analysis were conducted with GC-MS, whereas carotenoids were analyzed with HPLC.

Conclusions

We were able to detect altogether 47 fatty acids, 29 sterols and 13 carotenoids in our samples. As expected, fatty acids and carotenoids differed on class-level, but sterol composition was more heterogeneous within class and did not improve the class-level separation. However, sterols provided additional information on the abundance of specific genera within a class. GC-MS analysis could not separate cyanobacteria from algae due to their high variation in fatty acid composition and the absence of sterols, but when adding carotenoids to PCA, cyanobacteria clustered better together than when using only fatty acids and sterols. We conclude that the accuracy of phytoplankton identification can be significantly improved by combining these three groups of chemotaxonomic biomarkers

FEMS7-0030
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CITRUS MICROBIOMES: THE UNTAPPED DIVERSITY AS A SOURCE TO MINE FOR BENEFICIAL MICROBES

R. Penyalver[1], M.A. Forner-Giner[2], L.F.W. Roesch[3]
[1]Instituto Valenciano De Investigaciones Agrarias Ivia, Centro De Proteccion Vegetal Y Biotecnologia, Moncada, Spain
[2]Instituto Valenciano De Investigaciones Agrarias Ivia, Centro De Citricultura Y Produccion Vegetal, Moncada, Spain
[3]Universidade Federal Do Pampa Unipampa, Cip-biotech, Sao Gabriel, Brazil

Backgrounds

Citrus are the most cultivated fruit crop worldwide. Commercial citrus trees are grafted plants, where rootstocks are the hub of the most importantly horticultural and pathological traits. Mutualistic microbes present in root-associated communities (rhizospheric plant microbiome) have a great impact on host physiology and health, then providing a potential source of a variety of benefits for their host.

Objectives

The present study was devoted to stablish (i) the structure, variation and assembly of the rhizospheric citrus microbiome of two rootstock genotypes; and (ii) a prevalent "core-citrus" microbiome, as well as, to identify "genotype-specific" members.

Methods

Exploring citrus microbiota for beneficial microbes is the first step to develop future biotechnological applications for citrus industry. However, to carry it out successfully, it is crucial to capture as much as possible bacterial diversity. Therefore, metagenomic approaches are used to uncover the broad diversity of plant-associated communities. Previous studies on rhizospheric citrus microbiota were done elsewhere, but as far as we know, this is the first study of citrus microbiomes based on deep sequencing of 16S rRNA gene amplicon libraries (metataxonomics). Here, we present a detail characterization of the ectorhizosphere-associated microbiome of two agronomically-distint citrus rootstock genotypes grown in one soil for over 20 years.

Conclusions

We not found a significant genotype-dependent fine-tune of bacterial communities, since any citrus rootstock genotype selected an specific bacterial community. Nevertheless, some differentially abundant "operational taxonomic unit" OTUs were found in higher proportions in one genotype, suggesting that this citrus rootstock genotype has higher affinity for certain bacteria. We identifed a prevalent "core-citrus" microbime comprisig 544 OTUs as highy citrus-adpated bacterial members selected by the host. In summary, the untapped diversity of rhizospheric citrus microbiomes has provided a potential source to mine for plant beneficial microbes.

FEMS7-1763
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CELL BOUND PROTEINS P40 AND P75 ARE MULTIFUNCTIONAL MURAMINIDASES TYPICALLY FOUND IN THE LACTOBACILLUS CASEI/PARACASEI/RHAMNOSUS TAXON

G. Abitayeva[1], S. Sosa Carrillo[2], A. Mencher Beltran[3], N. Navarro Lleo[3], J.M. Coll[3], C. Bäuerl[3], G. Perez Martinez[3]
[1]L.N. Gumilyov Eurasian National University, Department of Natural Sciences, Astana, Kazakhstan
[2]Center for Research and Interdisciplinarity CRI, Center for Research and Interdisciplinarity, Paris, France
[3]Instituto de Agroquímica y Tecnología de Alimentos CSIC, Food Biotechnology, Paterna, Spain

Backgrounds

Lactobacillus casei proteins p40 and p75 (CmuA and CmuB) belong to a large family of cell wall proteins that contain a carboxy(C)-terminal CHAP or NLPC/P60 domain and with a variety of biological functions related to the nature of their amino(NH)-terminal domain. In Firmicutes they are frequently found secreted in the medium or bound to the cell wall with autolysin/muraminidase activity.

Objectives

The aim of this work was to study the Phylogeny and dissemination of this family of proteins and their likely (eco)physiological role.

Methods

Initially, PCR for specific conserved regions in their genes and western blot assays with polyclonal antibodies against their non-conserved NH-terminus regions of p40 and p75 showed that their presence is very specific of the taxon *L. casei/paracasei/rhamnosus*. Under laboratory conditions, these proteins had been found attached to the cell wall and also secreted in the culture medium. Using the mentioned antibodies, their presence was also detected in fermented milk commercial products that contained probiotic strains of *L.casei*. In order to partially explain their mode of action from the outer side of the cell wall, his-tag purified soluble proteins were used to complement satisfactorily p40 and p75 deficient mutants, and they also showed a significant cell lysis activity on different Firmicutes like, *Staphylococcus aureus, Enterococcus faecalis* and *Listeria monocytogenes*.

Conclusions

Results indicate p40 and p75 are found only in *L.casei/paracasei/rhamnosus* taxon and these secreted proteins can mediate the correct conformation of the cell wall, their presence in the extracellular medium may result in a competitive advantage.

FEMS7-2470
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE SPERMOSPHERE EFFECT: BUILDING UP PLANT MICROBIOME ASSEMBLY

J.E. Pérez-Jaramillo[1], V.J. Carrión[1], M. de Hollander[1], R. Mendes[2], J.M. Raaijmakers[1]
[1]Netherlands Institute of Ecology NIOO-KNAW, Microbial Ecology, Wageningen, The Netherlands
[2]Embrapa Environment, Laboratory of Environmental Microbiology, Jaguariúna, Brazil

Backgrounds

Plants have a significant influence on the diversity and activity of soil microbial communities. During imbibition and germination, plant seeds release chemically diverse exudates thereby promoting microbial activity in the zone surrounding the seed, also referred to as the spermosphere. To date, little is known about the diversity and activities of microbial communities in the spermosphere and how this short-lived plant developmental stage affects microbiome assembly.

Objectives

Here, we deciphered the magnitude of the spermosphere effect for two different food crops, i.e. tomato and bean. More specifically, we investigated if a plant genotype-dependent influence is discernible in the spermosphere and to what extent the spermosphere microbiome relates to the rhizosphere microbiome.

Methods

We selected wild and modern accessions of tomato and common bean for which strong differences in the rhizosphere microbiome were found in previous studies.

Conclusions

Community profiling of the spermosphere revealed a decrease of α-diversity of all crop accessions as compared to the bulk soil. Similarly, a significant difference in the β-diversity was observed between bean accessions and bulk soil. Bacteroidetes, Firmicutes and Proteobacteria were the bacterial phyla that consistently responded to the seed germination and were significantly more abundant in the spermosphere. Albeit small, significant differences in the β-diversity were detected between wild and modern crop accessions, suggesting a plant genotype-dependent effect already at this early developmental stage. If and how seed exudates are the main driver of the differences in spermosphere communities between the wild and modern crop accessions is currently under investigation.

FEMS7-1021
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BACTERIAL COMMUNITIES AMPLICON PROFILES MODELLING BY MARKOV CLUSTERING AND NEURAL NETWORKS. DEFINING REFERENCE ANNOTATION SIGNATURES

D. Pérez-Villarroya[1], *A. Moya*[2,3,4], *G. D'Auria*[1,3]
[1]*FISABIO-Public Health, Sequencing and Bioinformatics Service, Valencia, Spain*
[2]*FISABIO-Public Health, Genomics and Health Area, Valencia, Spain*
[3]*Centro de Investigación en Red en Epidemiología y Salud Pública, CIBEResp, Madrid, Spain*
[4]*Institut Cavanilles de Biodiversitat i Biologia Evolutiva ICBiBE- Universitat de València, Evolutionary Genetics Unit, Valencia, Spain*

Backgrounds

Association studies between taxonomy contingency tables and related metadata describing the origins or the state of the samples, are commonly carried out inferring statistical models. This process is usually carried out in a closed framework where only subject samples are statistically described in terms of case vs controls, physiological conditions, environmental/metabolic differences, etc.. While more and more amplicon surveys as well as metagenomic datasets are made available from almost all known environments, multiple sample comparisons methods and platforms become a hot issue to resume and categorize datasets towards biological comprehension.

Objectives

We can use already published and/or new datasets to create annotation profile models by the mean of supervised machine learning techniques. Thus, we can then classify new samples on the basis of the proper annotation signatures.

Methods

In this work we employed unsupervised Markov cluster algorithm (mcl) to enrich authors provided metadata with formal samples categories. Neural networks (NNt) supervised machine learning techniques are then used to generate models from 16S taxonomy distributions suitable for further *de-novo* samples classification.

Conclusions

We found that using samples metadata coupled with mcl algorithm to define formal categories improve the NNt modeling showing higher sensitivity and specificity to classify samples. The combination of mcl and NNt is a powerful approach to create databases of profile-models for further classification of new samples enriched with new profiles when the issued sample is not recognized.

FEMS7-1776
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHARACTERIZATION OF BACTERIA ISOLATED FROM PROPOLIS

E. Casalone[1], F. Vitali[1], R. Alberti[1], I. Petrocelli[1], B. Perito[1]
[1]University of Florence, Department of Biology, Sesto Fiorentino Florence, Italy

Backgrounds

Propolis, an ancient natural remedy with antimicrobial and other therapeutic properties, is a resinous material derived by worker bees from the leaf buds and exudates of numerous trees. Bees used it to cover hive walls, seal openings and embalm invader insects, then contributing to the hive hygiene. Despite many studies recently done on microbiota of the hive system, especially of adult bee gut, very little is known about microbiota of propolis and its contribution to the hive health.

Objectives

First objective is to verify the presence of viable bacteria into propolis and identify them; secondly we want to investigate if bacteria would play any role in therapeutic properties of propolis. Here we report a preliminary study on the isolation and characterization of bacteria from propolis collected from the apiary of our Department.

Methods

We set up a method for recovering prokaryotic cells from propolis and isolated them by standard microbiological procedures. Isolated microorganisms were classified on the basis of colony and cellular morphology. Morphotypes were tested for their growth and antimicrobial properties, and identified by 16S rDNA analysis.

Conclusions

By analysing different samples of propolis we obtained up to 10^3 CFU/gram of propolis. Nineteen different bacterial morphotypes were recognized. Representatives for each morphotype differ in their spectrum of antimicrobial activity against Gram positive and Gram negative bacteria, and fungi, and in their ability to grow on aqueous extract of propolis as the only carbon source. Isolates identification is in progress.

Work supported by the Italian MIUR (Prot. 2012RCEZWH)

FEMS7-1143
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BACTERIAL PROLIFERATION AS AN ADAPTIVE STRATEGY UNDER STARVATION

O. Petrova[1], V. Gorshkov[1,2], A. Daminova[1], M. Ageeva[3], I. Sergeeva[4], E. Kovtunov[1], N. Gogoleva[1], Y. Gogolev[1,2]
[1]Kazan Institute of Biochemistry and Biophysics - RAS, Laboratory of Molecular Biology, Kazan, Russia
[2]Kazan Federal University, Biochemistry and Biotechnology, Kazan, Russia
[3]Kazan Institute of Biochemistry and Biophysics - RAS, Laboratory of Microscopy, Kazan, Russia
[4]Kazan Federal University, Genetics, Kazan, Russia

Backgrounds

To adapt to adverse conditions, bacteria stop their growth to divert resources in order to promote survival. The adaptive reactions are known to be controlled at the population level in cell density-dependent manner. Herewith, the way of bacterial adaptation at low population densities remained unknown.

Objectives

We attempted to clarify if bacteria are able to adapt to stress conditions at low population densities and if so, to characterize the specific features of such kind of adaptation.

Methods

Various wild type and mutant gram-negative and gram-positive bacteria were subjected to carbon (or nitrogen) starvation at high (10^6-10^9, above quorum level) and low (10^1-10^5, below quorum level) population densities and the resultant model cultures were monitored by means of CFU and genome copy counting, electron microscopy, gene expression analysis and assessed for their resistance to various stress factors (cross-protection effect).

Conclusions

Bacteria are able to adapt to starvation at low population density; herewith, the initial part of stress response is related to cell proliferation until the population density reaches 1 million cells/ml – the value at which intercellular communication as well as stringent response was induced. Herewith, the cells acquired specific morphology different from that of the cells starved at high density. As a result of adaptation, cells that starved at low density same as those starved at high density acquired cross-protected phenotype, e.g. became resistant to multiple stressors. Thus, depending on population density bacteria realize alternative adaptive strategies that enable their survival under stress conditions. This study was supported by RSF (15-14-10022).

FEMS7-0937
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

NOVEL ACETOBACTERIUM MALICUM STRAIN CAPABLE OF USING METALLIC IRON AS SOLE ELECTRON DONOR

J. Philips[1], E. Monballyu[1], S. Georg[1], K. Rabaey[1], J.B.A. Arends[1]
[1]Ghent University, Center for Microbial Ecology and Technology CMET, Ghent, Belgium

Backgrounds

Some microorganisms are capable of using metallic iron as electron donor. These strains cause severe corrosion to metallic structures, but can also have interesting biotechnological applications. Microbial electrosynthesis (MES) is an innovative technology to convert electrical power into biofuels. MES depends on homoacetogens capable of deriving electrons from a cathode to reduce carbon dioxide to acetate. Both metallic iron and a cathode are solid state electron donors, therefore, the use of metallic iron as electron donor for enrichments and isolations seems a promising strategy to obtain novel strains for microbial electrosynthesis.

Objectives

The goal of the presented work was to isolate acetogenic strains using metallic iron as electron donor from environmental rust samples.

Methods

The rust layer of metallic garbage present in a local river was scraped off and used to set up enrichments. Metallic iron particles were added as sole electron donor and methanogenic growth was inhibited. Isolation was performed using agar plates containing iron powder.

Conclusions

Most isolates were strongly related (99%) to *Acetobacterium malicum*. The new strain produces acetate 50 faster times (electron equivalent base) compared to hydrogen evolution due to abiotic corrosion. Consequently, this strain must have a mechanism for direct electron uptake or to enhance abiotic hydrogen evolution. Insights into the electron uptake mechanisms, as well as its capacity for microbial electrosynthesis will be presented. The study of this novel *Acetobacterium malicum* strain leads to new insights into biocorrosion and extracellular electron uptake mechanisms that can contribute to the improvement of microbial electrosynthesis.

FEMS7-3027
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ENHANCED MICROBIAL GROWTH AND METABOLISM IN PRESENCE OF SUSPENDED MINERAL PARTICULATES AND PROPOSED MECHANISMS

F. Picardal[1], *T. An*[1]
[1]*Indiana University, School of Public and Environmental Affairs, Bloomington, USA*

Backgrounds

Microbial interactions with fine inorganic particles in heterogeneous environmental systems can positively or negatively affect microbial growth and metabolism.

Objectives

We systematically examined mechanisms to explain observed microbial growth enhancement by particles.

Methods

We investigated the effects of hydrous ferric oxide (HFO) and Min-U-Sil 5, a high-purity silica, on *Acidovorax* sp. 2AN growth in batch cultures.

Conclusions

These micron- and submicron-sized particles stimulated growth and substrate utilization of strain 2AN in both oxic and anoxic cultures, although inhibition was initially observed with HFO. When grown anaerobically on acetate and nitrate in the presence of 1 mM HFO, final protein concentrations were 24.6% higher with HFO, compared to cultures lacking HFO, although 2AN is not an Fe(III)-reducer. Compared to non-amended controls, anaerobic growth in the presence of Min-U-Sil 5 was more rapid and 16% more protein was produced at the end of the experiment. Under aerobic conditions, protein concentrations were 15% and 13% higher in cultures with Min-U-Sil 5 and HFO, respectively, than in controls lacking particulates. Strain 2AN also formed more pili when grown with Min-U-Sil 5 than in its absence. Growth enhancement did not result from particulates serving as an electron acceptor, nutrient source, or pH buffer. Enhanced growth and metabolism was also not due to surface-charge-associated changes in proton motive force and increased ATP generation. The stimulatory effect may be the result of greater microbial access to sorbed substrates or a more generalized effect on gene expression, as evidenced by increased pili formation during particulate-cell association.

FEMS7-2079
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INTEGRATION OF MOLECULAR AND ISOTOPIC ANALYSIS TO DEFINE AEROBIC AND ANAEROBIC PATHWAYS OF IN-SITU BIODEGRADATION OF MONOCHLOROBENZENE

I. Pietrini[1], F. de Ferra[2], G. Carpani[2], T. Stella[3], A. Franzetti[3], I. Gandolfi[3], M. Marchesi[1], L. Alberti[1], L. Zaninetta[4]
[1]Politecnico di Milano, Department of Civil and Environmental Engineering,
Piazza Leonardo da Vinci 32- 20133- Milan, Italy
[2]Istituto Eni Donegani Environmental Technologies, Research Center for Non-Conventional Energy,
Via Maritano 26- 20097 San Donato Milanese- San Donato Milanese- Milan, Italy
[3]Università di Milano-Bicocca, Department of Earth and Environmental Sciences,
Piazza della Scienza- 1- 20126- Milan, Italy
[4]Syndial S.p.A, Environmental Business Services,
Piazza M. Boldrini- 1 – 20097 S. Donato Milanese- Milan, Italy

Backgrounds

Bacterial communities associated with contaminated sites represent a great opportunity for environmental bioremediation considering that bacteria are able to use a wide number of chemical compounds as a source of carbon and energy. The use of an integrated approach based on different methodologies to gather more information about site-specific potential for bioremediation is gaining a wider acceptance from public authorities.

Objectives

The main objective of our work was to define quantitative indicators to assess the intrinsic degradation potential of a Monochloroebenzene (MCB)-contaminated aquifer by the use of a “toolbox” based on isotopic and molecular biology analyses.

Methods

Microcosms with groundwater collected from a MCB-contaminated site were set up under aerobic and anaerobic conditions to simulate both natural attenuation and biostimulated degradation processes. Enrichment factors for ^{13}C were determined by Compound Specific Isotope Analysis (CSIA). High-throughput sequencing (Illumina) and Ion Torrent analysis and quantitative PCR were performed to gain insights on the structure of the microbial community and to identify functional biomarkers.

Conclusions

MCB was completely depleted upon addition of nutrients, in both the conditions. Concerning aerobic biodegradation, CSIA results confirmed negligible C isotope fractionation under oxidative conditions. The gene *tod*, coding for toluene dioxygenase and *Pseudomonas* genus were identified as molecular and taxonomic marker, respectively. Moreover, preliminary results indicate that the anaerobic degradation of MCB is not linked to *Dehalobacter* or *Dehalococcoides* driven reductive dechlorination. On-going experiments suggest that the involved genes share considerable sequence homology with genes present on the plasmid pBHB of *Comamonas sp.*

FEMS7-3037
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

FLUORESCENCE REPORTER GENE PLATFORM APPLICABLE FOR THE DETECTION AND QUANTIFICATION OF HORIZONTAL GENE TRANSFER IN ANOXIC ENVIRONMENTS

R. Pinilla[1], *S. Sørensen*[1], *L. Riber*[1]
[1]*University of Copenhagen, Biology Microbiology Section, København, Denmark*

Backgrounds

The study of horizontal gene transfer (HGT) in microbial communities has been revolutionised by advances in cultivation-independent methods based on fluorescence reporter-gene technologies. However, the use of fluorescent markers like green fluorescent protein (GFP) and mCherry is limited by environmental constraints that affect the correct maturation of their fluorophores, such as oxygen availability and pH levels. Few studies have focused on elucidating their impact, and the sheer amount of distinct protein variants requires each system to be examined in an individual fashion. The wealth of ecologically and clinically relevant oxygen-deprived micro-habitats in which bacteria thrive, calls for the urgent development of suitable tools that permit their study.

Objectives

Development of an aerobic fluorescence recovery method for mCherry and GFPmut3, as well as characterisation of the impact the pH has on their fluorescence intensities. Validation of the dual-labelling system for the study of HGT in anoxic milieus.

Methods

The time-course fluorescent recovery of mCherry and GFPmut3 *in vivo*, as well as the effect of the extracellular pH on fluorescence was monitored through flow cytometry. The applicability of the findings was validated in anaerobic filter mating experiments.

Conclusions

The findings present a solution to an intrinsic problem that has long hampered the utilisation of this system to study HGT in environments devoid of oxygen, highlight its pH limitations, and provide the experimental tools that will help broaden its horizon of application to other fields.

FEMS7-0466
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

METAVIR-ALP: ALPINE LAKES BENTHIC VIRAL COMMUNITY STRUCTURE AND DIVERSITY: A METAGENOMIC AND ECOLOGICAL APPROACH. A PILOT STUDY.

F. Pinto[1], N. Salmaso[2], N. Segata[1]
[1]University of Trento, CIBIO, Trento, Italy
[2]Fondazione Edmund Mach, Sustainable ecosystems & bioresources, San Michele all' Adige, Italy

Backgrounds

After three decades since the first virus-like particles (VLP) were observed, the viral role in aquatic ecosystems is becoming clearer. By impacting bacterial communities, viruses influence evolutionary dynamics, mineralisation processes, energy transfer across trophic levels and greenhouse gas emission. Nonetheless, virus in freshwater systems are yet a neglected component.

Objectives

The MetaVir-Alp project proposes a multidisciplinary approach combining metagenomics, microbiology, and advanced computation to characterise the genetic diversity, structure and function of viral and bacterial communities in lakes sediment and water. Several Alpine lakes will be investigated along an altitudinal gradient that mimics a natural temperature gradient.

Methods

In this pilot investigation, we reported the taxonomic, genotypic and functional diversity of the viral and bacterial community within one Alpine lake. We established a well-defined protocol for sample collection and preparation in order to get the best yield of the extracted viral genetic material from sediments. Water at three depths and different aliquots of sediments were collected at Caldonazzo Lake and processed separately. Bacterial and viral DNA were subjected to Illumina HiSeq 2000.

Conclusions

Results provided novel insights into the viral and bacterial genetic diversity of freshwater ecosystems. We also observed substantial variability of the community structure and diversity along the vertical environmental gradient, in the water column and sediments. This pilot study was crucial for the development of the MetaVir-Alp project where microbial diversity will be investigated over a temporal and altitudinal dimension to appraise the potential influence of anthropogenic impacts and global warming on Alpine lakes.

FEMS7-0908
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DIVERSITY OF FUNGAL COMMUNITIES IN DEAD LOGS: EFFECTS OF FOREST STRUCTURE AND SUBSTRATE QUALITY IN FOUR UNMANAGED ITALIAN FORESTS

S. Pioli[1], A. Bani[1], L. Borruso[1], L. Brusetti[1], R. Tognetti[2]
[1]Free University of Bolzano, Faculty of Science and Technology, Bolzano, Italy
[2]Università del Molise, Biosciences and Territory, Pesche, Italy

Backgrounds

Wood-inhabiting fungi are considered the major decomposers of organic matter in forest environments playing a crucial role for the maintenance of critical ecosystem processes. Despite their importance, very few studies have investigated wood-inhabiting fungi with molecular techniques, especially in Southern Europe.

Objectives

In our study, substrate quality along with stand structural attributes and ecological parameters were used to disentangle the relationships between habitat characteristics and fungal community structure.

Methods

Along the Italian peninsula, we collected dead wood samples from four forest reserves that differed in species composition, management history and successional stage. The fungal community was investigated through fingerprinting analysis while wood physical properties and stand features were assessed in the field. In addition, lignin, carbon and nitrogen content were analysed for each sampled wood.

Conclusions

Our results revealed an influence of stand structural characteristics in differentiating the fungal communities across the reserves, in particular, in relation to dead wood volume and living trees species diversity. Moreover, the mean annual temperatures exerted a major role compared to other climatic variables (i.e. precipitation), while only C:N ratios, decay stage and wood diameter resulted significantly correlated with the overall community structure regarding wood characteristics. We conclude that forest structural and compositional heterogeneity have strong effect on communities of decomposer fungi, suggesting that the promotion and conservation of complex ecosystems, with different tree species and great dead wood availability, is essential for the preservation of fungal diversity and forest functioning.

FEMS7-1602
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ECOLOGY OF LISTERIA MONOCYTOGENES IN SOIL: EFFECT OF THE BIOTIC ENVIRONMENT ON SURVIVAL AND TRANSCRIPTOME RESHAPING.

L. Gal[1], A. Ortiz Camargo[2], D. Garmyn[3], P. Piveteau[4]
[1]AgroSup Dijon, INRA UMR1347 Agroécologie, Dijon, France
[2]Univ. Bourgogne Franche Comté, INRA UMR1347 Agroécologie, Dijon, Colombia
[3]Univ. Bourgogne Franche Comté, INRA UMR1347 Agroécologie, Dijon, France
[4]Univ. Bourgogne Franche Comté, INRA UMR1347 Agroécologie, France, France

Backgrounds

Listeria monocytogenes is a bacterium found in many habitats such as soil, plants, animals, foodstuff and food processing facilities. Circulation between habitats is a source of its transmission to food and eventually to the consumer. Contaminated foodstuff is indeed the vector of listeriosis, a life-threatening disease mainly to immunocompromised people and pregnant women. One of the intriguing facets of *Listeria monocytogenes* is its ability to adapt its physiology to complex, heterogeneous habitats. With its complex chemistry, texture, dense microbiota and overall biotic fraction, soil is a nice example of such heterogeneous habitat. Persistence in many habitats suggests that *L. monocytogenes* is able to integrate a range of environmental cues in the circuitry of regulation of transcription.

Objectives

This study aimed at assessing in one hand extrinsic factors that shape the fate of *L. monocytogenes* in soil, and in the other hand the response of *L. monocytogenes* to the biotic environment found in soil.

Methods

The response of *L. monocytogenes* EGD-e to the biotic fraction of soil was investigated in irradiated and untreated microcosms through a combination of transcriptomic approaches and population dynamics.

Conclusions

The fate of *L. monocytogenes* is dependent on both abiotic and biotic characteristics and the latter have a major impact on the dynamics of the populations of *L. monocytogenes* in soil. Major transcriptome reshaping was observed where *L. monocytogenes* recruits its repertoire of transporters and specific pathways to access and utilise the available substrates. The biotic environment further affects transcriptome and triggers further regulation.

FEMS7-0391
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHOLESTEROL CONVERSION INTO COPROSTANOL BY BACTERIAL STRAINS ISOLATED FROM THE HUMAN GUT MICROBIOTA CAN MODIFY HOST CHOLESTEROL METABOLISM

A. Potiron[1], A. Kriaa[1,2], S. Boudebbouze[1], A. Bruneau[1], M. Lhomme[3], P. Lesnik[3], E. Maguin[1], P. Gérard[1], M. Rhimi[1]
[1]INRA-Institut National de la Recherche Agronomique, MICALIS, Jouy en Josas, France
[2]LBPPE, Centre de Biotechnologie, Sfax, Tunisia
[3]INSERM, UMR S939, Paris, France

Backgrounds

Cholesterol level management is a keystone to limit cardiovascular diseases. Gut microbiota has emerged as a new target to modulate host metabolism including cholesterolemia. In fact, the transformation of cholesterol into coprostanol by intestinal microorganisms was reported almost one century ago. Interestingly, human feces analyses revealed two sub-populations: i) high-converters –HC– subjects showing a complete or almost complete conversion of cholesterol to coprostanol and ii) low-converters –LC– individuals showing no or low conversion level. We confirmed this distribution on a human cohort. However, the impact of this gut bacterial metabolism on blood cholesterol remains unclear. Only one cholesterol-converting bacterial strain has been isolated from human feces.

Objectives

Our objective was to isolate new coprostanol-producing bacteria and to evaluate *in vivo* their potential impact on cholesterolemia.

Methods

We isolated 27 new cholesterol-converting bacteria from HC feces using different cholesterol-supplemented culture media. Five bacteria were selected on their coprostanol production features, growth rate and biomass production. Two were retained for their abilities: i) to colonize germ-free mice gastro-intestinal tract and ii) to produce coprostanol *in vivo*. Germ-free mice associated with these bacterial strains will receive a cholesterol-enriched diet for 10 weeks. Cholesterolemia and *in vivo* production of coprostanol will be followed as well as expression of genes involved in cholesterol metabolism and inflammation.

Conclusions

We expect that these bacterial strains may limit the increase in cholesterolemia thanks to cholesterol-to-coprostanol conversion in the gut.

FEMS7-1388
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIODEGRADATION AND DETOXIFICATION OF NICOSULFURON HERBICIDE BY NEW ISOLATE OF PSEUDOMONAS SP. IN A CONTAMINATED AGRICULTURE SOIL

A.A. Pourbabaee[1], *A. Solymani*[1], *H. Alizadeh*[2]
[1]*University College of Agriculture and Natural Resources- University of Tehran, Soil Science, Karaj, Iran*
[2]*University College of Agriculture and Natural Resources- University of Tehran, Department of Agronomy and Plant Breeding, Karaj, Iran*

Backgrounds

Nicosulfuron, a selective systemic herbicide from the sulfonylurea group, is used as post emergence for controlling weeds in corn fields.

Objectives

To being toxicity due to inhibitory effect on acetolactate synthetase enzyme, persistence of the herbicide residues in soil, and its adverse effects of the herbicide, this research were aimed to study the biodegradation and detoxification of it by bacterial isolates.

Methods

In this study, ten bacterial strains from the soil treated with herbicide during several years were isolated and screened on mineral base medium. Among ten isolated assayed for detoxification activity, the strain B9 was the most effective isolate and was chosen as the superior strain. Based on 16S rRNA sequence analysis and phenotypic characteristics, the isolate B9 was closely (96%) related to *Pseudomonas aeruginosa* BWH05. The effect of the herbicide on some biological traits in soil was measured. These traits were increased in less time in soil treated with herbicide as compared to non-inoculated soil.

Conclusions

Results of obtained from assay of nicosulfuron concentration in liquid medium by HPLC analysis showed that B9 isolate could reduce nicosulfuron concentration up to 61% and 90% for 5 and 30 days respectively at 30˚C . The results of experiments Petri dish and greenhouse bioassay of treated herbicide by B9 isolate on *Lepidium sativum* showed that this strain was able to detoxify and significantly reduced the inhibition effect of the herbicide. Therefore, this isolate can be used for removal of herbicide residues in contaminated soils.

FEMS7-1612
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

REMEDIATION OF PHENANTHRENE FROM A SALT- AFFECTED SOIL IN MICROCOSM SYSTEM BY CONSORTIUM OF HALOTOLERANT BACTERIA

A.A. Pourbabaee[1], *N. Hashemi*[1], *M. Farahbakhsh*[1], *G. Bagheri marandi*[1]
[1]*University College of Agriculture and Natural Resources- University of Tehran, Soil Science, Karaj, Iran*

Backgrounds

Salinity is one of the major problems for bioremediation of petroleum-contaminated soils around of refineries Iran. Although in recent years many PAH biodegradation studies have been done but the major of reports are in aquatic system and or non-saline soils. So an acceptable process for remediation of salt-affected soil contaminated to PAHs is need.

Objectives

The aim of this study was to evaluate the performance of few native strains of halotolerant bacteria able to degrade phenanthrene (as model of PAH), in a saline soil in a microcosm system under aeration.

Methods

To conduct this study, the activity of bacteria in the form of quaternary in 24 flask containing 50 grams of salt-affected soil (by the electrical conductivity of 30) and phenanthrene at a concentration of 500 ppm under two conditions aeration and without aeration at 80 % moisture holding capacity and in 28 °C incubation for 90 days was investigated. The bacteria used in this research were: *Halobacillus debanensis* QSH1, *Bacillus hewajnpoensis* QSH3, *Acidovorax delafieldii* QSH12 and *Bacillus rhizosphera* QSH14, which recently isolated from saline soil and has been demonstrated phenanthrene degradability in mineral aquatic medium. Analysis of treatments has been done with measuring respiratory rate, dehydrogenase activity and HPLC of soil which extracted in N-hexane.

Conclusions

The results of the experiments indicated that consortium of 4 halotolerant bacteria used in this study, more than 90% of phenanthrene after 15 days under aerated conditions has been removed.

FEMS7-2977
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DUAL RNA-SEQ UNEARTHED THE UNSUCCESSFUL RESPONSE OF A PHYTOPATHOGENIC OOMYCETE TO THE ANTAGONISTIC STRATEGIES IMPLEMENTED BY THE BIOCONTROL BACTERIUM LYSOBACTER CAPSICI AZ78

G. Puopolo[1], *S. Tomada*[1], *P. Sonego*[1], *M. Moretto*[1], *K. Engelen*[1], *I. Pertot*[1], *M. Perazzolli*[1]
[1]*Fondazione Edmund Mach, Research and Innovation Centre, San Michele all'Adige, Italy*

Backgrounds

The establishment of plant diseases also depends on the outcome of the complex microbial interactions occurring between pathogenic microorganisms and their antagonists residing in the rhizosphere.

Objectives

The molecular mechanisms activated in the interaction between the beneficial rhizobacterium *Lysobacter capsici* AZ78 and the soilborne phytopathogenic oomycete *Phytophthora infestans* were finely dissected using a dual transcriptomic approach.

Methods

Simultaneous transcriptional changes of both *L. capsici* AZ78 and *P. infestans* occurring after 6 and 24 h of interaction were analysed. The transcriptional profiling of *L. capsici* AZ78 was mainly characterized by the up-regulation of genes involved in the biogenesis of type 4 pilus and lytic enzymes (cellulases, glucanases and proteases)involved in the host colonization and the subsequent attack of *P. infestans* cell wall, respectively. The activation of detoxification processes allowed *L. capsici* AZ78 to overcome the defence activity of *P. infestans*. Moreover, genes deputed to the antibiotic biosynthesis were up-regulated in *L. capsici* AZ78 resulting in the up-regulation of genes involved in programmed cell death in *P. infestans*. The consequences of the activation of these processes resulted in the overall down-regulation of primary metabolic pathways in *P. infestans*, such as carbohydrate, nucleic acids and protein metabolisms.

Conclusions

The dual transcriptional analysis revealed that the antagonistic activity of *L. capsici* AZ78 is based on transcriptional activation of motility, attachment, lytic and antibiotic processes. On the other hand, the activation of programmed cell death in *P. infestans* could explain its inability to actively respond to *L. capsici* AZ78 attacks.

FEMS7-2544
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SPATIAL AND TEMPORAL DYNAMICS OF ANTIBIOTIC RESISTANCE GENES IN THE URBAN WATER CYCLE

M. Quintela-Baluja[1], *D. Graham*[1]
[1]*Newcastle University, Environmental Engineering, Newcastle Upon Tyne, United Kingdom*

Backgrounds

Antibiotic **r**esistance (AR) is an evolutionary response in bacteria to antibiotics, which has become a serious worldwide public health concern with **A**ntibiotic **R**esistance **G**enes **(ARGs)** now considered by many as emerging pollutants. However, researchers have been focused on identifying and understanding the most important antimicrobial resistance mechanism in clinical settings. Although this data is useful in patient treatment, these knowledge is not able to wholly explain the spread of clinical relevant ARGs on a global scale.

Objectives

Our project studied the ARGs dynamics through the Urban Water Cycle, improving understanding of the main genetic transitions at different compartments.

Methods

High-throughput quantitative PCR targeting 285 ARGs, 8 transposases, and 16S ribosomal RNA gene was used in samples collected at different seasons, and different catchments of the UWC (Clinical, Domestic, wastewater treatment plant, and river). The microbial community was obtained by amplification of the 16S rRNA on the Ion Torrent PGM. Additionally, a specific set of primers and probes were designed to study the integron gene cassette rearrangement.

Conclusions

Our results suggest a spatial and temporal dynamic of the ARGs not only in their abundance, but also in the number of antibiotic resistance mechanisms. Additionally, we postulate that the clinical integron 1 is behind the genetic transition of ARGs in the UWC due to: i) its high dynamic (specially in the activated sludge), ii) its high relative abundance, iii) its function as a carrier of ARGs, and iv) its presence at different bacterial species (plasmid DNA or in chromosomal DNA)

FEMS7-2135
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHROMIUM REDUCTION CAPABILITY BY NATIVE FUNGI ISOLATED FROM BIOSOLIDS OF A WASTEWATER TREATMENT PLANT

L.F. Quiroz Salazar[1], V.A. Castañeda-monsalve[1], O. Ruiz-Villadiego[1], O.I. Montoya-Campuzano[1]
[1]Universidad Nacional de Colombia, Science, Medellín, Colombia

Backgrounds

Heavy metals are contaminants commonly found in the environment and Chromium is one of the top twenty environmental contaminants. Chromium has five valence states but the most toxic form is the one with valence VI (Cr6+), while the less toxic form has valence III (Cr3+). For instance it would be useful to transform chromium to its reduced form (Cr3+), but the anthropogenic activities increase chromium concentration in soil and water sources.

In order to reduce this contaminant, researchers have developed some physicochemical techniques but these have shown low efficiency and may promote formation of harmful substances.

Biological techniques like bioremediation have demonstrated to be an efficient way and environmental friendly. These techniques involve the use of native microorganisms isolated from contaminated sources and test their ability to reduce heavy metals.**Objectives**

The aim of the project was to isolate native fungi capable to reduce chrome in high concentration.

Methods

During this study 15 fungi were isolated from samples of biosolids; in addition 9 were isolated from the air. Microorganisms were cultured in PDA media with addition of a known concentration of hexavalent chromium. The minimal inhibitory concentration was determined, and the reduction ability was tested in ME broth with hexavalent chromium at 800 ppm, 100 rpm and room temperature; one sample each 24 h was taken, hexavalent chromium was measured by spectrophotometry using the diphenilcarbazide method and total chromium by atomic emission.

Conclusions

The obtained isolates from polluted environmental samples showed a notable chromo-reductase activity. An unreported chromo-tolerant specie was found.

FEMS7-3097
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

TOLERANCE OF INDIGENOUS TRICHODERMA STRAINS TO INCREASED CONCENTRATIONS OF METALS

G. Racić[1], I. Vukelić[1], M. Lazovic[2], L. Jovanovic[1], L. Nešić[3], M. Vrvić[4], D. Panković[1]
[1]Educons University, Faculty of ecological agriculture, Sremska Kamenica, Serbia
[2]A BIO TECH LAB, A BIO TECH LAB, Sremska Kamenica, Serbia
[3]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia
[4]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia

Backgrounds

Fungi belonging to the *Trichoderma* genus are known for a wide range of beneficial properties which are the basis of their application in different branches of biotechnology. Bioremediation is ecologically friendly method that is used to alleviate heavy metal pollution of the environment. Indigenous microorganisms are considered as most suitable for successful bioremediation for the particular site and metal. Thus, this study was performed with previously identified indigenous *Trichoderma* isolates.

Objectives

The aim of this study was to determine diethylene triamine pentaacetate (DTPA)-extractable metal content from different soil types in Vojvodina region and compare it with total metal content. Based on the calculated ratio of extractable/total metal content, sites where anthropogenic origin of metals were defined. Indigenous *Trichoderma* isolates from the selected sites where used for *in vitro* tolerance test to cooper and nickel.

Methods

The concentrations of extractable metals in the soil samples were determined by inductively coupled plasma-optima emission spectrometry (ICP-OES), Thermo iCAP 6500 Duo System. The metal tolerance test was performed by measuring the diameter of fungal colonies on plates containing Ni(II) and Cu(II) amended potato dextrose (PDA) medium at final concentrations of 0.5, 1, 2 and 4 ppm, and comparing the diameter with colonies on control PDA plates.

Conclusions

The ratio of DTPA-extractable in total metal content was the highest for Cu and Ni in the examined soil samples, indicating that contamination with these metals is of anthropogenic origin. Strains that showed best tolerance to the examined concentrations are proposed to be tested as potential bioremediation agents.

FEMS7-1355
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE CLEANUP OF A RIVER: MICROBIAL TAXONOMIC AND FUNCTIONAL FEATURES INVOLVED IN THE TIETE RIVER SELF-PURIFICATION REVEALED BY METAGENOMICS

L.R.M. Rada[1], E.G. Silva[1], S. Ichiwaki[1], L.R. Olchanheski[1], W.L. Araújo[1]
[1]Institute of Biomedical Sciences, Microbiology, Sao Paulo, Brazil

Backgrounds

Tietê River in Brazil is the most important river in São Paulo State and also one of the most polluted rivers of the country. Interestingly, even with the daily dumping of domestic and industrial sewage, it doesn't remain polluted. Upon leaving the city of São Paulo the river begins a self-purification process and, at its mouth, water can be considered pristine.

Objectives

What's the role of the microorganisms in the self-purification process? How the environmental conditions affect it? To unveil these questions, four contrasting sites along the river were chosen for metagenomic analyses to characterize the taxonomic and functional diversity of microorganisms, and try to understand their role in the river self-purification process.

Methods

Metagenomic Shotgun sequencing of water samples was performed using Illumina HiSeq™ platform. Unassembled DNA sequences were annotated using the Metagenomics Rapid Annotation Server (MG-RAST) and statistical analyses were performed using the Statistical Analysis of Metagenomic Profiles (STAMP) software package.

Conclusions

Clear differences were found in taxonomic and functional profiles between the sampling sites, mainly related to the water quality. Prevalence of *Gamma* and *Betaproteobacteria* as well as genes involved in anaerobic organic matter degradation was observed in the contaminated sites. In non-polluted sites, genes related to iron acquisition and photosynthesis were predominant, along with high abundance of *Cyanobacteria*.
Our results suggest an important influence of local environmental features in the microbial community structure. Also our data showed that, in different sites of the river, self-purifying mechanisms are mostly related to microbial metabolic processes, mainly associated to organic matter degradation.

FEMS7-2785
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL COMMUNITIES IN THE NESTS OF BORNEO'S 'EXPLODING ANTS' COLOBOPSIS CYLINDRICA COMPLEX

M.J. Rahimi[1], C. Pretzer[1], M. Fischer[2], A. Kopchinskiy[1], A. Laciny[3], M. Grujic[1], K. Abu Salim[4], L. Lim[5], C.M. Chan[5], A. Parich[2], R. Schuhmacher[2], H. Zettel[3], I.S. Druzhinina[1]
[1]Vienna University of Technology,
Institute of Chemical Engineering- Research Area Biotechnology and Microbiology, Vienna, Austria
[2]University of Natural Resources and Life Sciences BOKU,
Department of Agrobiotechnology- Center for Analytical Chemistry, Tulln, Austria
[3]Natural History Museum Vienna, Zoological Department, Vienna, Austria
[4]Universiti Brunei Darussalam, Environmental and Life Sciences, Bandar Seri Begawan, Brunei
[5]Universiti Brunei Darussalam, Chemistry Programme, Bandar Seri Begawan, Brunei

Backgrounds

Cavity nesting 'exploding ants' in the *Colobopsis cylindrica* species complex ('COCY', Hymenoptera: Formicidae) possess hypertrophied mandibular gland (MG) reservoirs containing a diversity of chemical compounds. Most evolutionary advanced COCY ants use these products in suicidal defense of territory (autothysis), but many species have no such behavior. Interestingly some substances detected in MG have been described as antimicrobials in particular with fungicidal activity. This indicates that COCY MG products either play a role in nest hygiene and/or are involved in a yet unknown ant-microbe interaction.

Objectives

In this study, we focused on the microbial diversity in natural nests of the two evolutionary, phenotypically and ecologically well distinct COCY species: YG and BBQ, respectively.

Methods

Conclusions

Nests of suicidal YG in dead branches of *Shorea johorensis* were colonized by generalist hypocrealean fungi such as *Trichoderma* spp. and *Xenoacremomiun* sp. with the minor occurrence of *Penicillium* spp., Mucoromycotina and Saccharomycetales fungi. The microbiome of BBQ ants that rarely exhibit autothysis, was drastically different as it was dominated by a novel dimorphic melanised 'black yeast' fungus from Pleosporales that grew in its yeasty form only inside the nest but not *in vitro*. The fungus was slowly growing and had remarkable antifungal potential. The bacterial diversity in nests of both species has been assessed by Illumina sequencing and consists of species with potential to fix atmospheric nitrogen. The results of the microbe-microbe interaction assays and the effect of COCY chemistry on isolated microorganisms will be discussed in light of possible roles of hypertrophied MG reservoirs in these ants.

FEMS7-0282
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

STUDYING THE EFFECTS OF ANAEROBIC BACTERIA IN CORROSION OF COOLING TOWER IN SANANDAJ AND BISTOON POWER PLANTS

B. Rahimian Zarif[1], *A. molasharifi*[1]
[1]*Department of biology- Sanandaj branch- Islamic Azad University- Sanandaj- Iran, biology, Sanandaj, Iran*

Backgrounds

In the age of technology ahead Power generation has a special strategic importance. Given that a few microns organisms have the capability to disrupt power generation, Identify these organisms in the industry has particular importance. Due to the structure of the cooling tower And the possibility of biofilm formation and the anaerobic area and aerotolerant properties of also some anaerobic bacteria (SRB), And their ability to corrosion of metals, these bacteria are the most damaging bacteria in the industry.

Objectives

In this study, anaerobic bacteria that influenced on cooling tower's corrosion were identified in Sanandaj and bistoon power plants

Methods

these bacteria were identified based on the reaction products with a medium composition. After anaerobic sampling and transferring, Samples were cultured in postgateB, And in anaerobic jars were incubated for 21 days. After 5 repetitions with an interval of one month. Thus in positive sample the black precipitate (hydrogen sulfide) from the reaction between medium and bacterial products was observed

Conclusions

The result of this reaction in Sanandaj power plant cooling towers for reasons such as structure and water supply were negative. While this reaction in a cooling tower Bistoon for reasons such as openness of water cycle and water supply have led to positive results. According to the results, with comprehensive study of environmental conditions and structure of the cooling towers, large losses can be avoided

FEMS7-2624
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

WORMS AND BACTERIA: TOOL TO UNRAVEL HOST-MICROBIOTA INTERACTIONS

N. Rai[1], *P.E. Olsson*[1], *J. Jass*[1]
[1]*Örebro University, The Life Science Centre- School of Science and Technology, Örebro, Sweden*

Backgrounds

The host-microbiota plays an important role in facilitating digestion, providing nutrients and energy as well as in human diseases. However it is difficult to establish whether changes in microbiota are the cause or effect of their associated pathologies. Recent studies have shown that the gut microbiota impacts all aspects of host's life including development, metabolism, immunity and behaviour. The use of the mouse model offers a solution but the complexity and cost limits the use of this mammalian model. *Caenorhabditis elegans* is an excellent model for studying processes in a whole organism including energy metabolism, immunity, ageing and other signalling pathways. It is therefore possible to use these worms to understand how the microbiota affect the host physiology. The bacterial diet largely influences the stress response, metabolism and lifespan of the *C. elegans.* Despite the potential importance, the microbiome of this model organism is largely unknown. Hence exploring the microbiota of *C. elegans* and studying the contribution of the environment and food source would improve our understanding of the role of microbes in host physiology.

Objectives

Our study aims to establish the implications of different pathogenic and lab strains of *Escherichia coli* on *C. elegans* associated gut microbiota.

Methods

Viability, lifespan and gene expression analysis will be used to explore the role different bacteria and environmental contaminants on *C. elegans* physiology

Conclusions

Our results show that the type of bacterial diet impacts the development of *C. elegans*, and this may be due to perturbation of the gut microbiota of the worms.

FEMS7-3193
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIOFILM FORMATION AND COLONIZATION OF PLANT TISSUES BY A PLANT-BENEFICIAL STRAIN PSEUDOMONAS DONGHUENSIS P482

M. Rajewska[1], *S. Jafra*[1]
[1]*University of Gdansk,*
Department of Biotechnology- Intercollegiate Faculty of Biotechnology- UG & MUG, Gdańsk, Poland

Backgrounds

Biofilm is the most frequently encountered form of bacterial growth in many ecosystems. Bacteria interact with various surfaces, forming complex, multicellular communities ensuring survival in specific environmental conditions. Numerous research is conducted to elucidate the relationship between the ability of bacteria to colonize biotic and abiotic surfaces. In some cases, a correlation between biofilm development and adhesion of bacteria to plant tissues (e.g. root surface) is observed. The *Pseudomonas donghuensis* P482 strain is a little-known isolate from tomato rhizosphere, exhibiting antimicrobial activity towards bacterial and fungal plant pathogens. Earlier studies demonstrated that P482 efficiently colonizes the plant rhizosphere, however, the mechanism underlying this phenomenon has not been solved up to date.

Objectives

The objective of the ongoing research is to identify and analyze factors (genetic and environmental) involved in the ability of P482 strain to form biofilm on abiotic surfaces and to colonize plant tissues.

Methods

The P482 strain and its knock-out mutants were analyzed for their ability to move on synthetic media. Biofilm formation on abiotic surfaces (polystyrene and glass) was assessed in varying conditions by crystal violet staining and fluorescence confocal microscopy. Colonization of plant tissues was analyzed in an *in vitro* culture system on maize seedlings.

Conclusions

The obtained preliminary results suggest possible correlation between the ability to form biofilm on abiotic surfaces and colonization of plant tissues by the P482 strain. Moreover, the strain exhibits variability of biofilm formation depending on the environmental conditions. Further studies on biofilm formation and plant root colonization are under way.

FEMS7-2553
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RHIZOSPHERIC BACTERIAL COMMUNITIES ASSOCIATED TO GOLDEN SAGUARO (NEOBUXBAUMIA POLYLOPHA), GROWING IN SEMI-DESERTIC AREAS IN CENTRAL MEXICO, AS ASSESED BY CULTURING AND DEEP SEQUENCING TECHNIQUES

H. Ramirez Saad[1], M.L. Daniel[2], D.L.T.H. María Eugenia[1], M.A. Francisco[3], A.G. Félix[4]
[1]Universidad Autonoma Metropolitana-Xochimilco, Sistemas Biologicos, Mexico City, Mexico
[2]Universidad Autonoma Metropolitana-Xochimilco, Maestría en Ciencias Agropecuarias, Mexico City, Mexico
[3]Estación Experimental El Zaidin-CSIC, Ecología de la Rizosfera, Granada, Spain
[4]Universidad Autonoma Metropolitana-Lerma, Ciencias Ambientales, Lerma de Villada, Mexico

Backgrounds

Semiarid areas in Queretaro State, Mexico represent the southernmost extension of the Chihuahuan Desert. This area has been nominated as natural reserve because of its unique plant biodiversity constituted mainly by xerophytes, with exceptional occurrence of rare and endemic species. This natural diversity is being threatened by human activities; however, plant spoliation is probably the main hazard to various cacti species. Management and propagation of cactus plants normally do not consider the microbial community associated to their roots, which probably are related to plant important processes. Introduction of nursery-propagated cacti into natural environments is often hampered by low survival rates, this could be due to a lack of specific bacteria associated to the roots of forestation individuals.

Objectives

To assess by a NGS approach, structure and composition of rhizosphere bacterial communities of golden saguaro considering as possible sources of variation: plant origin and plant size.

Methods

N.polylopha individuals growing in 2 natural stands and in a nursery were selected, and sampled according to 2 size ranges: small (<1m tall), medium (1-6m tall). Rhizospheric samples were collected and used for metagenomic DNA extraction and further massive sequencing on an Illumina platform.

Conclusions

Variation sources have little effect on the rhizospheric bacterial community composition at Family and Class level. Differences in structure and composition were found at Genus level, however, the proportion of unclassified sequences was greatly increased in all samples.

FEMS7-0117
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CELL DIVISION BY LONGITUDINAL SCISSION IN THE INSECT ENDOSYMBIONT SPIROPLASMA POULSONII

E. Ramond[1], C. Maclachlan[2], S. Clerc-Rosset[2], G.W. Knott[2], B. Lemaitre[1]
[1]EPFL, Global Health Institute, Lausanne, Switzerland
[2]EPFL, Interdisciplinary Center for Electron Microscopy CIME, Lausanne, Switzerland

Backgrounds

Most bacteria rely on binary fission, which implies length growth followed by transversal division. So far, examples of bacteria with a Y-shape longitudinal scission remain scarce.

Objectives

Spiroplasma poulsonii is a natural endosymbiont of *Drosophila. It persists in the hemolymph (insect blood) of females and is vertically transmitted (from mother to offsprings). Spiroplasma has a distinctive helical morphology and belongs to the class of Mollicutes, a group of bacteria devoid of cell wall.* Here, we investigated the mechanism of *Spiroplasma poulsonii* division.

Methods

We used scanning and transmission electron microscopies, and immunogold staining to analyze *S. poulsonii* cell division. *S. poulsonii were collected from freshly hemolymph sample of Drosophila.*

Conclusions

Our study shows that *Spiroplasma* was not only found as long helical filaments, as previously described, but was also found in a Y-shaped form. The use of electron microscopy and immunogold staining of the FtsZ protein unambiguously linked the *S. poulsonii* Y shape to cell division. Observation of the Y shape in another *Spiroplasma*, *S. citri*, and anecdotic observations from the literature suggest that cell division by longitudinal scission might be prevalent in the *Spiroplasma* clade. Our study is the first to report the Y-shape mode of cell division in an endosymbiotic bacterium and adds *Spiroplasma* to the so far limited group of bacteria known to utilize this cell division mode. It also raises the hypothesis that this mode of cell division by longitudinal scission could be linked to the symbiotic mode of life of these bacteria.

FEMS7-1718
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SAMPLER SELECTION FOR WHOLE AIRBORNE BIOLOGICAL COMMUNITY ANALYSIS USING SHOTGUN NGS

A. Rastrojo[1], A. Núñez[2], A.M. García[3], D.A. Moreno[2], A. Alcamí[1]
[1]Centro de Biología Molecular Severo Ochoa, Virology, Madrid, Spain
[2]Escuela Técnica Superior de Ingenieros Industriales Universidad Politécnica de Madrid, Departamento de Física Aplicada e Ingeniería de Materiales, Madrid, Spain
[3]Escuela Técnica Superior de Ingenieros Industriales Universidad Politécnica de Madrid, Escuela Técnica Superior de Ingenieros Industriales, Madrid, Spain

Backgrounds

A great effort is being made by the international community to reduce air pollution but very little is known about the biological community (airbiota) present in the outdoor air. The study of the airbiota is relevant due to its potential role in dissemination of plant, animal and human diseases, with important implications in public health, and a huge economic impact on crops and stockbreeding productivity. Our current knowledge about airbiota is almost restricted to pollen and fungal spores, measured through microscopy techniques, and bacterial communities studies using culture-dependent analysis or amplicon-based approaches, while studies on viruses are almost inexistent. Therefore, a global overview of the airborne biological community is crucial to understand the dynamics of the air ecosystem, to identify marker organisms and to establish new air quality indicators. However, there is not a standarized method to capture the whole diversity present in the air. Therefore, the evaluation of different capturing methods is a key step to establish a standard framework.

Objectives

The present work aims to establish a standard method for airborne particles capture.

Methods

We have tested simultaneously different methods for collecting airborne organisms, including impactor samplers, liquid impingers, cyclonic samplers and different filters. Additionally, to avoid the biases of culture-dependent methods or amplicon-based analyses, we have performed a next generation sequencing (NGS) shotgun approach (Illumina) to uncover the whole airborne diversity.

Conclusions

A detailed analysis of the genetic diversity captured using different methodologies and the taxonomic classification of the biota in the air will be presented.

FEMS7-3195
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DEVELOPMENT OF A MICROBIAL PROCESS OF METHANE GENERATION FROM COAL AT THERMOPHILIC CONDITIONS

R. Rathi[1], L. Meeta[1], N. Singh[2], P. Sarma[2], P. Kishore[2], B. Lal[1]
[1]The Energy and Resources Institute- New-Delhi, Environmental and Industrial Biotechnology, New-Delhi, India
[2]ONGC ENERGY CENTER, Biotechnology, New-Delhi, India

Backgrounds

There has been a growing interest in coal bed methane (CBM) both for energy production and reduction of greenhouse gases. CBM has been used as an alternative fossil resource. India has CBM generating potential but lacks in technology for *in-situ* biogenic methane generation from its coal reservoirs. most of the unminable coal seams/reserves that have CBM recovery potential are at a higher temperature range.

Objectives

The objective of the current study is to develop a thermophilic methanogenic consortium from samples collected from high temperature coal seams with CBM potential. Also, optimization of parameters that may subsequently influence the *in situ* biogenic CBM production by selected thermophilic methanogenic consortium.

Methods

In this study, a thermophilic methanogenic consortium was enriched from samples collected from Banaskantha coal drilling mines (depth of about 1200m) of western India that had bottom -hole temperature of 60 °C. Microbes were enriched with 1% (w/v) bituminous coal obtained from the same coal mines. Subsequently, effect of reservoir parameters were optimized for enhanced CBM generation for the selected consortium CBM 4.

Conclusions

The methane production from coal was observed by the thermophilic methanogenic consortium CBM 4 at temperature 60 °C, pH 7.5 and salinity 0.1% NaCl. This study suggested that the selected consortium isolated from Banaskantha coal mines is capable of utilizing high rank bituminous coal as a carbon-energy source at thermophilic condition. Thus, indicating a possibility of stimulating or augmenting this consortium in coal seams of similar temperature and to develop a microbial process for enhanced CBM generation.

FEMS7-0552
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IMPACT OF BONE ENVIRONMENT ON STAPHYLOCOCCUS AUREUS BIOFILM FORMATION

F. Reffuveille[1], Q. Vallé[1], L.G. Thiery[1], J. Josse[2], S.C. Gangloff[1]
[1]University of Reims Champagne Ardenne, BIOS, Reims, France
[2]University of Lyon 1, INSERM U1111 - CNRS UMR5308, Lyon, France

Backgrounds

As discovered during the last decades, most of the bacteria are able to form “biofilm” representing the bacterial capacity to build a multicellular structure highly resistant to any concentration of antimicrobial compounds. Despite the unbelievable medical progress, the success of all surgical interventions is threatened by the development of biofilm-associated infections. They represent 65 to 80% of nosocomial infections and *Staphylococcus aureus (S. aureus)* is the leading species in this domain.

Objectives

Bacteria can detect any stress (like presence of antibiotics) in order to survive and biofilm could be one of those “resistance” strategies. However, the stress signals that could induce irreversible adhesion and so the bacterial biofilm program, is still unknown. In consequence, there is a real challenge to better understand this phenomenon.

Methods

In this study, regarding more specifically at the context of bone infections, collected supernatants of osteoblast-like cells (Saos-2) were applied on *S. aureus* culture. Bacterial adhesion was evaluated after 24h of contact using two models to apprehend any signal from osteoblasts cell culture that could induce or influence biofilm first step formation. Osteoblast cell culture supernatants increased adhesion capacity by 2-fold ($P<0.001$) compared to medium control in our two models.

Moreover, different molecules as magnesium or iron, present in bone environment, were tested. Among others, high concentration of magnesium and iron increased the biofilm formation.

Conclusions

In conclusion, there is an impact of bone environment on biofilm formation. The perspectives of this work will be to determine the most important compounds that leads to the formation of the biofilms.

FEMS7-0992
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

QUORUM SENSING INHIBITION MECHANISMS IN SEA ANEMONES AND HOLOTHURIANS MICROBIOTA

J.C. Reina[1], E. León-Palmero[2], I. Reche[2], J. Martín[3], I. Pérez-Victoria[3], F. Reyes[3], I. Llamas[1,4]
[1]University of Granada, Department of Microbiology, Granada, Spain
[2]University of Granada, Department of Ecology, Granada, Spain
[3]Fundación Medina,
Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Armilla, Spain
[4]Institute of Biotechnology- Center for Biomedical Research, Armilla, Spain

Backgrounds

Aquaculture is one of the most expanding industries in the world, as reported by the Food and Agriculture Organization of the United Nations (FAO) in its last report in 2016. Nevertheless, it has to face different problems including infections, some of which are caused by microorganisms, which are the main cause of severe economic losses in this sector.

Recently, it has been shown that the expression of virulent genes in some bacterial pathogens is controlled by a population-density-dependent gene-expression mechanism known as quorum sensing (QS). The inhibition of QS systems has been demonstrated to be a potential strategy in the fight against diseases affecting aquaculture. Here, we present a screening of the production of compounds with QS inhibition activity amongst the bacteria isolated from the microbiota of marine invertebrate animals, an underexplored source of bioactive molecules.

Objectives

The aim of this study is the selection of compounds that interfere with QS systems (quorum quenching enzymes, QQ, and quorum sensing inhibitors, QSIs) of bacteria isolated from the microbiota of two sea anemones and two holothurians.

Methods

A high-throughput screening of quorum sensing inhibition activity amongst 856 strains was carried out. The selected QQ strains were then evaluated against a broad range of QS signal molecules, whilst the selected QSI strains were tested against the QS systems of *Chromobacterium violaceum* and *Pseudomonas aeruginosa*.

Conclusions

We have selected 31 symbiotic bacteria from marine invertebrate animals with quorum sensing inhibition mechanisms that are based on enzymatic degradation and interference of the detection of signal molecules.

FEMS7-1024
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHARACTERIZATION OF AHL-DEGRADING ENZYMES FOUND IN STENOTROPHOMONAS MALTOPHILIA STRAINS ISOLATED FROM HOLOTHURIA SPP AND ANEMONIA SULCATA

J.C. Reina[1], J. Martín[2], I. Pérez-Victoria[2], N. Ruiz[1], F. Reyes[2], I. Llamas[1,3]
[1]University of Granada, Department of Microbiology, Granada, Spain
[2]Fundación Medina,
Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Armilla, Spain
[3]Institute of Biotechnology- Center for Biomedical Research, Armilla, Spain

Backgrounds

The quorum sensing (QS) signal molecules *N*-acylhomoserine lactones (AHLs) control the expression of virulence factors in some Gram-negative pathogenic bacteria. The use of antibiotics in aquaculture is creating an increased resistance and consumers are rejecting their use.

One of the most promising tools for fighting infections that affect aquaculture is based on the disruption of QS through a mechanism known as quorum quenching (QQ). In this work we present the study of four *Stenotrophomonas maltophilia* strains isolated from the microbiota of invertebrate marine animals such as *Holothuria* spp and *Anemonia sulcata* that have the ability to degrade AHLs as mechanism of QQ.

Objectives

Characterization of the AHL degradation activity of four *S. maltophilia* strains.

Methods

The QQ activity of the four *S. maltophilia* strains was tested against a broad range of AHLs and monitored in well-diffusion agar-plate assays by using the indicator strains *Chromobacterium violaceum* CV026, *C. violaceum* VIR07 and *Agrobacterium tumefaciens* NTL4 (pZLR4). The enzymes were expressed in *Escherichia coli* and their AHLs degradation activity was confirmed through a well-diffusion agar-plate and HPLC-HRMS analyses.

Conclusions

We have found that *S. maltophilia* environmental strains isolated from marine invertebrates degrade a wide range of AHLs, including synthetic and natural AHLs produced by different microorganisms through enzymes with homology to penicillin acylases.

FEMS7-1981
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL SULFONAMIDE DEGRADATION: FURTHER INSIGHTS INTO THE METABOLIC PATHWAY AND IMPLICATIONS FOR WASTEWATER TREATMENT

A.C. Reis[1,2], M. Čvančarová[2], K. Kroll[2], B.A. Kolvenbach[2], A. Timm[3], R. Peschke[3], M. Majewsky[4], H.P.E. Kohler[5], P.F.X. Corvini[2], O.C. Nunes[1]
[1]Faculty of Engineering of University of Porto, Chemical Engineering - LEPABE, Porto, Portugal
[2]Fachhochschule Nordwestschweiz FHNW,
Hochschule für Life Sciences - Institut für Ecopreneurship, Basel, Switzerland
[3]Karlsruhe Institute of Technology KIT, Engler-Bunte-
Institut- Bereich Wasserchemie und Wassertechnologie, Karlsruhe, Germany
[4]Heidelberg University Hospital, Clinical Pharmacology and Pharmacoepidemiology, Heidelberg, Germany
[5]Swiss Federal Institute for Aquatic Science and Technology Eawag, Environmental Microbiology, Dübendorf, Switzerland

Backgrounds

Sulfonamides are widespread micropollutants that carry unpredictable risks to diverse ecosystems. Despite being ubiquitous and persistent, these antibiotics are often degraded by diverse microbial communities. Nevertheless, the full metabolic pathway has only been proposed for a few strains and its relevance for wastewater treatment has not been assessed.

Objectives

In this study, we aimed at characterizing the metabolic pathway for sulfamethoxazole (SMX) degradation in *Achromobacter denitrificans* PR1 and at evaluating whether these lab-based findings are valid in the wastewater treatment process.

Methods

LC-MS/MS was used to identify the metabolites produced by strain PR1 and to monitor the concentration of 8 sulfonamides and 13 metabolites in five wastewater treatment plants. Radio-labeling was used for benchmarking assays of activated sludge samples.

Conclusions

Degradation of SMX by strain PR1 resulted in the accumulation of 3-amino-5-methylisoxazole and revealed new dead-end products resulting from the parallel mono-hydroxylation of the aniline moiety at *ipso* and *ortho*/*meta* positions and additional molecular rearrangements. The metabolites found in wastewater samples suggest this to be a prevalent pathway for sulfonamide elimination

Furthermore, culturing PR1 for 14 days revealed a slow growing Gram positive bacterium (GP). Based on the 16S rDNA analysis, this strain showed highest sequence similarity (96.75%) to *Leucobacter luti* RF6T, indicating affiliation to the phylum *Actinobacteria*. Strain GP grows only in co-culture with PR1 and SMX degradation also only occurs in the same conditions, suggesting a possible metabolic cooperation between the strains.

Our results give valuable insights at different levels of microbial ecology of sulfonamide degrading strains.

FEMS7-1993
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL DIVERSITY AND FUNCTIONALITY IN AVOCADO RHIZOSPHERE SOILS AMENDED WITH COMMERCIAL BIOFERTILIZERS

R. Prabhaharan[1], S. González[2], M. Astorga-Eló[3], M. Jorquera-Tapía[4]
[1]Laboratory of Applied Microbial Ecology, Universidad de La Frontera, Temuco, Chile
[2]Doctoral Program in Natural Resources Sciences, Universidad de La Frontera, Temuco, Chile
[3]Doctoral Program in Natural Resources Sciences, Universidad de La Frontera, Temuco, Chile
[4]Scientific and Technological Bio-resource Nucleus, Universidad de La Frontera, Temuco, Chile

Backgrounds

The perception of eating healthy, avocado (Persea americana) consumption and demand hits highest growth concentration in different world markets. Chile is one of the largest producer and exporter of avocado in world market. Currently, the production is significantly decreased from 263,476 to 164,720 ton ha^{-1} (2009-2013), which mainly attributed to climatic events in association with global warming. Many studies have revealed the protective mechanisms of plant growth promoting microorganisms used as biofertilizers to promote plant crops against diverse environmental stresses. In Chile,the existing biofertilizers are imported from various countries and sold by local distributers. Studies revealed that the inoculation of foreign microorganisms can disrupt the native soil microorganisms associated with plant crops. Certainly, native strains are disappeared from the microbial community structure, it is highly difficult or even impossible to detect, and, thus, they can possibly affect certain plant physiological functions.

Objectives

To explore the effect of commercial biofertilizers on the diversity and functionality of microbial communities in rhizosphere soils of avocado.

Methods

The effect of commercial biofertilizers on the rhizosphere microbial communities (bacteria, fungi and archaea) of avocado have been studied by denaturing gradient gel electrophoresis (DGGE) analysis using ribosomal genes as target. The functional diversity has been determined by using miniaturized kits Biolog®Ecoplates (Biolog, Inc.; US).

Conclusions

This study has clearly determined which imported commercial biofertilizer had significant effect on microbial communities in rhizosphere soil of avocado. This evidence can be very useful when imported biofertilizer based management practices are being designed, revised and/or established in commercial avocado nursery soils.

FEMS7-1148
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

VERTICAL TRANSMISSION OF BACTERIA IN THE INTRAUTERINE ENVIRONMENT

A. Rey-Mariño[1], A. Vallés[1], N. Jiménez-Hernández[1], F. Ballester[2], M.J. Gosalbes[1], M.P. Francino[1]
[1]Centro Superior de Investigación en Salud Pública,
Unidad Mixta de Investigación en Genómica y Salud, Valencia, Spain
[2]FISABIO-UJI-University of Valencia, Epidemiology and Environmental Health Unit of Research, Valencia, Spain

Backgrounds

The origin of the bacteria present in the intrauterine medium remains not defined or fully studied. The complexity of the treatment and quantification of DNA from placenta and meconium, due to the low microbial content of these environments, makes research in this field difficult. In addition, possible contamination should be considered for the taxonomic assignment of these samples.

Objectives

The aim of this project is to define maternal microorganisms that are transmitted to offspring through the placenta and are accumulated in meconium during intrauterine life.

Methods

We used massive sequencing of 16S rRNA gene amplicons in paired samples of placenta and meconium microbiota in 23 term newborns from a Spanish birth cohort and performed taxonomic assignment and estimation of diversity for each pair of samples obtained. In order to detect a possible transmission of bacteria from placenta to meconium, we performed clustering analysis based on taxa composition and abundance to determine similarities between samples and searched for 100% identical 16S rRNA sequences in paired meconium/placenta samples.

Conclusions

We detected 100% identical 16S rRNA sequences in placenta and meconium from 21 of the 23 pairs analysed, with *Lactobacillus* as the most abundant genus among the transmitted bacteria. Our findings suggest that the meconium microbiota has an intrauterine origin as a possible vertical transmission of bacteria between mother and child was detected. These bacteria will be further studied as potential candidates for being used as probiotics that promote a successful programming of health at immune and metabolic levels.

FEMS7-2053
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

LARGE SCALE ANALYSIS OF THE TOPOLOGY AND DYNAMICS OF METABOLIC NETWORKS OF ENDOSYMBIOTIC BACTERIA OF INSECTS

M. Reyes Prieto[1], D.J. Martínez Cano[2], C. Vargas Chávez[1], L. Delaye[2], A. Moya[1,3]
[1]Institute for Integrative Systems Biology I2SysBio - University of Valencia, Evolutionary Genetics, Valencia, Spain
[2]Cinvestav Unidad Irapuato, Genetic Engineering, Irapuato- Guanajuato, Mexico
[3]Valencian Region Foundation for the Promotion of Health and Biomedical Research FISABIO, University of Valencia, Valencia, Spain

Backgrounds

More than 100 genomes of endosymbiotic bacteria of insects have been sequenced. Through their analysis we are aware that they present different degrees of genome degradation, a common feature of bacteria becoming endosymbiotic, dependent on the age of the symbiotic formation and the evolutionary pressures acting upon their genes. In free living organisms, metabolic networks are extremely robust to the deletion of genes. Network modulation can evolve rapidly through horizontal gene transfer thus complementing these gene deletions. In endosymbiotic bacteria, the behavior of metabolic networks is exceptional, since once the bacteria becomes endosymbiotic, not only the loss of genes is important and accelerated, but they are unable to receive new genetic information due to the barriers imposed by the intracellular lifestyle.

Objectives

Analyses on metabolic networks of different organisms have helped understand the principles under which these respond and accommodate to varying conditions. We focus on large-scale topology and dynamics of metabolic networks of endosymbiotic bacteria to understand the evolution of these topologies and the elements involved in the adaptation to endosymbiotic life.

Methods

Using bioinformatic tools, we developed a pipeline for large-scale analysis of metabolic networks which allows us to build complete networks of each endosymbiotic bacterial genome, compare between these endosymbiotic networks and an assembled metabolic network from a theoretical minimal cell, and analyze the topology of each organism.

Conclusions

We found that as the genomes of endosymbiotic bacteria of insects shrink, their metabolic networks lose robustness, showing different topologies dependent on the different stages of genome degradation.

FEMS7-1335
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION AND DIVERSITY OF ACTINOMYCETES FROM MARINE COASTAL SEDIMENT

I. Ribeiro[1,2,3], *D. A. M. Alexandrino*[1], *R. S. Oliveira*[4,5], *C. Santos*[1], *F. Pereira*[1], *A. P. Mucha*[1], *M. F. Carvalho*[1]

[1]*Interdisciplinary Centre of Marine and Environmental Research- University of Porto Terminal de Cruz eiros do Porto de Leixões- Avenida General Norton de Matos- S/N- 4450-208, N/A, Matosinhos, Portugal*

[2]*Faculdade de Ciências- Universidade do Porto- Rua do Campo Alegre- s/n- 4169-007, FCUP, Porto, Portugal*

[3]*Instituto de Ciências Biomédicas Abel Salazar Universidade do Porto- Rua de Jorge Viterbo Ferreira- 228- 4050-313, ICBAS, Porto, Portugal*

[4]*Research Centre on Health and Environment- School of Allied Health Sciences- Polytechnic Institute of Porto- Rua Dr. António Bernardino de Almeida- 400- 4200-072 Porto- Portugal, Environmental Health, Porto, Portugal*

[5]*Centre for Functional Ecology- University of Coimbra- Calçada Martim de Freitas- 3000-456, Life Sciences, Coimbra, Portugal*

Backgrounds

Environmental pollution is a serious problem for ecosystems and human health. Many pollutants are associated with several diseases and with the deterioration of human health. In addition to this, the increasing consumption of pharmaceuticals is also becoming an environmental problem leading, among other effects, to the emergence of antibiotic resistant microorganisms.

As health problems pose a constant threat to humanity, it is necessary to continuously seek for new therapeutic solutions, one of which is the discovery of new bioactive compounds.

Actinomycetes are a group of Gram-positive and filamentous bacteria well known for their unmatched capacity to produce a vast range of bioactive compounds with high industrial and pharmaceutical interest.

Objectives

The objective of this study was to investigate the diversity of actinomycetes associated with marine costal sediment and to explore their potential to produce bioactive compounds.

Methods

The isolates were obtained from coastal sediment collected in Parque Natural do Litoral Norte, Esposende, Portugal. To improve the isolation efficiency of actinomycetes, three different pre-treatments were applied and three selective culture media were used.

Conclusions

A total of 117 isolates were obtained from the collected marine sediment sample. 16SrRNA gene sequencing results revealed that most isolates belong to the *Micromonospora* genera. Some non-actinomycetes isolates have also been obtained, essentially belonging to the phylum Firmicutes and to the genera *Paenibacillus* and *Bacillus*, which also encompass many bacterial producers of interesting bioactive compounds. These isolates are presently being studied for their capability to produce bioactive compounds.

Acknowledgements: Investigator FCT, NORTE-01- 0145-FEDER- 000035 (ERDF), and ICBAS,University of Porto.

FEMS7-3012
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

STUDIES ON THE BIODEGRADATION OF THREE STRUCTURALLY RELATED ALIPHATIC ORGANOFLUORINES WITH ENVIRONMENTAL RELEVANCE

D. A. M. Alexandrino[1], I. Ribeiro[1,2,3], L. M. Pinto[4], R. Cambra[4], R. S. Oliveira[4,5], F. Pereira[6], M. F. Carvalho[1]
[1]Interdisciplinary Centre of Marine and Environmental Research, EcoBioTec, Matosinhos, Portugal
[2]Institute of Biomedical Sciences, University of Porto, Porto, Portugal
[3]Faculty of Sciences, University of Porto, Porto, Portugal
[4]Research Centre on Health and Environment, Department of Environmental Health, Porto, Portugal
[5]Centre for Functional Ecology, Department of Life Sciences, Coimbra, Portugal
[6]Interdisciplinary Centre of Marine and Environmental Research, BBE, Matosinhos, Portugal

Backgrounds

The use of synthetic organofluorines has significantly increased in the last decades, contributing to the emergence of many of these compounds in the environment. Fluoroacetates, mono-, di- and trifluoroacetate are aliphatic organofluorines with various uses in industrial applications. They have been reported to occur in the environment, mainly in aquatic compartments. Current biodegradation data indicate that these compounds are highly recalcitrant, with the exception of the monofluorinated molecule, thus justifying more studies on their biodegradation. Moreover, the wide industrial applications of fluoroacetates may lead to their simultaneous presence in the environment, being also important to understand how mixtures of these compounds influence their biodegradation.

Objectives

This work aimed to investigate the aerobic biodegradation of mono-, di- and trifluoroacetate as sole carbon sources, in mixtures and in cometabolism with acetate.

Methods

Biodegradation of fluoroacetates was studied in batch mode under aerobic conditions, using various environmental samples as microbial inocula. Different conditions were tested: biodegradation of fluoroacetates as sole carbon sources, mixtures of monofluoroacetate with di- or trifluoroacetate and mixtures of di- or trifluoroacetate with acetate as a cometabolite. Cultures were sampled at regular intervals for monitoring fluoride release and biomass growth. Degrading microorganisms were identified through 16S rRNA gene sequencing analysis.

Conclusions

A total of 13 bacterial strains isolated from distinct environmental sources had the capacity to biodegrade monofluoroacetate. Di- and trifluoroacetate were shown to be recalcitrant in all tested conditions. When in mixture, difluoroacetate was found to inhibit the metabolism of monofluoroacetate, which was not verified with trifluoroacetate.

FEMS7-1483
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

GROWTH IN A MULTISPECIES BIOFILM RESULTS IN REMODELLING OF CENTRAL METABOLISM

S. Rice[1], K.W.K. Lee[1], H.A.S. Nair[1], R.B.H. Williams[2], S. Kjellberg[1]
[1]Nanyang Technological University,
The Singapore Centre for Environmental Life Sciences Engineering, singapore, Singapore
[2]The National University of Singapore,
The Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore

Backgrounds

Mixed species biofilms have been shown in several studies to outperform mono-species biofilms. Here, a mixed species biofilm model comprising *Pseudomonas aeruginosa*, *Pseudomonas protegens* and *Klebsiella pneumoniae* was previously shown to display enhanced biofilm development and tolerance to antimicrobials. However, the mechanisms behind these traits are unknown.

Objectives

To define how mixed species communities interact to display emergent biofilm properties

Methods

RNA-sequencing was thus performed for mixed and mono-species biofilms subjected to different treatments (sodium dodecyl sulfate (SDS) treated and untreated), and for biofilms at different developmental stages (4, 5 and 6 days of growth) to identify key genes that account for these emergent traits.

Conclusions

It was found that *K. pneumoniae* and *P. protegens* cooperate through carbon metabolism, where genes associated with the pentose phosphate pathway and citric acid cycle were significantly altered. These results indicate the exchange of metabolites and improved resource utilization within the mixed species biofilms. Subsequently, it was demonstrated that *K. pneumoniae* grew slowly on citrate as a mono-species cultures, but achieved significant biomass when grown in the presence of *P. protegens* and *P. aeruginosa*. Despite having access to identical resources, a communal metabolic pathway that enables increased biomass production relative to mono-species biofilms is proposed. Comparison between SDS treated and untreated biofilms showed that production of SDS hydrolase (SdsA1) by *P. aeruginosa* increased 72 fold. The secretion of SdsA1 could explain the cross protection of SDS sensitive *P. protegens* in the mixed species biofilms. Therefore, these data demonstrate that synergistic interactions occur within mixed species biofilms, whereby the community members can share and redistribute resources to optimise their fitness.

FEMS7-2472
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ECOLOGICAL ASPECTS OF BACTERIAL HCN PRODUCTION

T. Rijavec[1,2], *A. Lapanje*[1,2,3]
[1]*Jozef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia*
[2]*Institute of Metagenomics and Microbial Technologies, -, Ljubljana, Slovenia*
[3]*Saratov State University, Remote Controlled Theranostic Systems Lab, Saratov, Russia*

Backgrounds

HCN producing bacteria (HPB) are commonly isolated from soil and predominantly identified as *Pseudomonas* spp. The ecological role of HCN is not always clear, though we show it is associated with mineral weathering and nutrient availability in the rhizosphere. Particularly interesting here is the oligotrophic alpine environment with its high ecological patchiness, which calls for detailed analysis of the spatial distribution of HPB populations, the biochemical characteristics and the evolutionary aspects of HCN production.

Objectives

We wanted to characterize the association of HPB with the rhizosphere of alpine pioneering plants and further examine for each strain in the population its ability to produce HCN. We wanted to determine how this ability is related to a specific rhizobacterial genotype. We finally wanted to characterize the genomes of few selected HPB, identify the *hcn* genes and assess the characteristics of gene regulatory elements.

Methods

We obtained a large collection of strains to characterize biochemically their ability to produce HCN and to genetically characterize each strain using BOX-PCR. We also characterized the production of HCN for selected strains during different growth phases. Using genome sequencing we characterized the *hcn* genes in few selected HCN producing strains.

Conclusions

The rhizosphere of pioneer plants is rich in HPB, harbouring the most potent HCN producers. Nevertheless, HCN+ phenotype is not associated with one particular genotype and it is still unclear how these genes are spread within the population. HPB genomes, diversity of *hcn* genes and regulation of HCN production will also be discussed.

FEMS7-2525
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

TYPE OF METABOLISM AND ATTACHMENT OF BACTERIAL CELLS PROMOTE HG ENTRY INTO THE ECOSYSTEM

T. Rijavec[1,2], M. Kovač Viršek[3], A. Lapanje[1,2,4]
[1]Jozef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia
[2]Instititute of Metagenomics and Microbial Technologies, -, Ljubljana, Slovenia
[3]Institute for Water of the Republic of Slovenia, -, Ljubljana, Slovenia
[4]Saratov State University, Remote Controlled Theranostic Systems Lab, Saratov, Russia

Backgrounds

Our understanding of bacterial mobilization of different forms of Hg is currently insufficient. More and more data shows that bacteria can mobilize also the inert mineral forms of Hg. Bacteria can solubilize mineral particles, which contain Hg using organic acids, siderophores, other secondary metabolites, extracellular proteins, etc. How efficiently Hg is released and whether it is transformed into the biologically available form by different bacteria has not been examined to date.

Objectives

In our study we wanted to test how different types of bacterial metabolism can affect the release of Hg from Hg-contaminated sediment. We also wanted to examine how the vicinity of cells and particles, and the attachment of cells to, can affects the increase of biological availability of Hg.

Methods

Using the *in vitro* whole-cell bacterial biosensor and measurements of total Hg, we assessed how different types of bacteria can mobilize Hg from Hg-contaminated sediment. Using bacterial culturing of strains collected from a Hg contaminated site and biochemical characterization, we assessed how the production of secondary metabolites can coincide with Hg mobilization and Hg resistance.

Conclusions

Certain bacteria, i.e. *Bacillus* spp., *Pseudomonas* spp., are more pronounced Hg mobilizers compared to other bacteria tested. Though the vicinity of cells and particles can be an important factor affecting Hg mobilization, certain extracellular secondary metabolites prove to be even more important in increasing the level of bioavailable Hg. Finally, ability to mobilize Hg might promote the spread of Hg resistance (*mer*), resulting in novel selective advantages.

FEMS7-1252
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IMPROVEMENT OF MAIZE GROWTH BY ROOT COLONIZING BACTERIA

C.H. Hernández Rodríguez[1], R.I. Arteaga Garibay[2], B. Rios Galicia[1]
[1]Instituto Politécnico Nacional, Microbiology, México city, Mexico
[2]Centro Nacional de Recursos Genéticos INIFAP, Microbiology, Tepatitlán- Jalisco, Mexico

Backgrounds

Microbial interactions with plants involve either endophytic or free living microorganisms able to establish interactions in the environment. Some associative and free-living microorganisms promote plant growth through a variety of mechanisms of biocontrol of pathogens, biofertilization or phytostimulation.

Objectives

The growth promoting plant-microbe interactions are studied for being a handful tool in the search of solutions that reduce crop problems through the biotechnological development of fertilizers.

Methods

A collection of 91 strains was isolated following a strategy of direct isolation and bacterial population enrichment. Plant-growth-promoting capabilities were measured and the most outstanding strains were selected for the assays in plants.

Conclusions

Already reported *Serratia marcescens, Ochrobactum anthropic* and *Gordonia terrae* strains were able to control fungal diseases caused by *Ustilago maydis, Fusarium culmorum* or *Sporisorium reillianum.* Meanwhile *Herbaspirillum seropedicae*, *Pantoea allii*, and *Phytobacter diazotrophicus* displayed plant-growth promotion capabilities such as nitrogen fixation, auxin production and phosphate solubilization. Despite observed effects and previous characterization of strains, it is still unknown the bacterial strategy that produce the effect *in vivo* due to the strains showed more than one growth-promoting strategy.

FEMS7-0857
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RHIZOBIUM ZEAE A NOVEL SPECIES WITH POTENTIAL AS PGPB FOR MAIZE CROPS

L. Celador-Lera[1], E. Menéndez[1], M.E. Velázquez[1], P.F. Mateos[1], R. Rivas[1]
[1]Universidad de Salamanca, Microbiología y Genética, Salamanca, Spain

Backgrounds

The members of genus *Rhizobium* are widely known as legume endosymbionts. Nevertheless, they have been also found in roots of non-leguminous plants, including some cereals such as rice or wheat, whose growth is promoted by these bacteria. Some of them have been firstly isolated from roots of cereals, such as *Rhizobium oryzicola,* which was isolated from rice roots. However, there is no evidence that new *Rhizobium* species have been isolated from maize roots.

Objectives

To evaluate the potential of *Rhizobium zeae* strain as a biofertilizer for *Z. mays* crops.

Methods

Here, a new bacterial species, designated *Rhizobium zeae*, was isolated from maize roots, which were collected in NW Spain. This strain produced siderophores and also, is an excellent producer of indole-acetic acid and/or derivatives. In vitro inoculation of *Z. mays* seedlings showed earlier root development than seedlings from the uninoculated treatment in the first stages of development. Fluorescence and confocal microscopy in vitro assays with the GFP-tagged strain showed that this strain interacts efficiently with maize roots, forming complex tridimensional structures and showing a special ability for colonizing maize tissues.Greenhouse assays were performed and the results confirmed the ability of this strain to promote maize growth, observing that the inoculated plants presented a superior number of knots and a significative increase of shoot length. A field trial was carried out, confirming that the inoculation of this strain contribute to an increased maize production.

Conclusions

The strain of *Rhizobium zeae* isolated from *Zea mays* is susceptible to be used as biofertilizer in these crops.

FEMS7-1235
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

METAGENOMIC ANALYSIS OF BIOREMEDIATION CONSORTIA.

R. Rivilla[1], D. Garrido-Sanz[1], J. Manzano[1], M. Redondo-Nieto[1], M. Martin[1]
[1]Universidad Autónoma de Madrid, Biología, Madrid, Spain

Backgrounds

Bioremediation of organic contaminants is carried out by individual microbia or by microbial consortia. Consortia are specially important when complex contaminants are tackled.

Objectives

To identify genes and pathways implicated in bioremediation of PCBs and diesel within the consortia

Methods

We have generated two microbial consortia by enrichment cultures from rhizospheric soil, using defined mineral medium supplemented with biphenyl or with diesel as the only carbon and energy sources. After several passes, consortia able to degrade either biphenyl (as an analogue of PCBs) or diesel were obtained. Samples of the cultures during exponential growth, were deep-frozen to be used as reference. DNA from these samples were used to analyze biodiversity, by means of sequencing and analysis of 16S libraries and to identify key genes and enzymes implicated in bioremediation by means of sequencing of metagenomics libraries.

Conclusions

Biodiversity analysis showed that the diesel consortium was more diverse than the biphenyl consortium (Shannon index 3.6 versus 3.0), although both of them contained a discrete (below 50) number of OTUS at the genus level. Both consortia were dominated by proteobacteria, especially pseudomonads but contained a significant number of OTUs from other phyla. Genera known by their ability to degrade PCBs such as *Pseudomonas, Rhodococcus, Achromobacter* and *Stenotrophomonas* were predominant in the biphenyl consortium, while *Pseudomonas*, *Azospirillum, Sphingobium* and *Chrysobacterium* dominated the diesel consortium. Metagenomic analysis is being used to identify genes and pathways relevant for bioremediation and to assign them to specific OTUs.

DGS was granted by FPU (FPU14/03965) from MECD, Spain. Funded by BIO2015-64480-R(MINECO/FEDER EU).

FEMS7-2837
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COMBINING DNA STABLE ISOTOPE PROBING AND METAGENOMICS TO EXPLORE THE PHYLOGENETIC AND METABOLIC MICROBIAL DIVERSITY IN ARSENIC CONTAMINATED SEDIMENTS

A. Rizoulis[1], *M. Héry*[2], *J.R. Lloyd*[3]
[1]*University of Central Lancashire, School of Forensic and Applied Sciences, Preston, United Kingdom*
[2]*Université Montpellier, Laboratoire HydroSciences Montpellier- HSM- UMR 5569 IRD- CNRS, Montpellier, France*
[3]*The University of Manchester,*
Williamson Research Centre for Molecular Environmental Science- School of Earth and Environmenta l Sciences, Manchester, United Kingdom

Backgrounds

The consumption of arsenic contaminated groundwater is causing a humanitarian disaster in Southeast Asia, threatening the health of tens of millions. The factors controlling arsenic release from sediments into groundwater have been the subject of numerous studies, which have shown that various geological, mineralogical and geochemical characteristics and processes can contribute to increased As(III) mobilisation. In addition, microbial reduction of As(V) sorbed to mineral surfaces is widely accepted as being an important mechanism of As(III) release in shallow anoxic aquifers.

Objectives

To date, molecular studies in arsenic contaminated environments have mainly described the phylogenetic diversity of the indigenous microbial communities. In this study, we combined DNA stable isotope probing and metagenomics in order to investigate the phylogenetic and metabolic microbial diversity in arsenic contaminated Cambodian sediments.

Methods

Following incubation with ^{13}C-lactate, the ^{13}C-labelled fraction of the DNA was isolated and after 454 pyrosequencing, more than 124,000 reads were obtained.

Conclusions

The results indicated the presence of a phylogenetically and metabolically diverse microbial community, which was dominated by members of the *Geobacter* genus. Metagenomic sequences related to respiratory arsenic reduction and lactate utilisation were also identified, as well as an increased number of sequences affiliated to motility, chemotaxis and *c*-type cytochromes. In addition, the identification of Archaeal sequences indicated the presence of metabolic interactions within the complex microbial community. The results from this study and the publicly accessible metagenome will allow comparisons to be made by future metagenomic studies in arsenic contaminated environments.

FEMS7-1078
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

STUDYING BACTERIAL CELL SURFACE PROPERTIES THROUGHOUT BIOFILM FORMATION ON DIFFERENT SURFACES

C.J. C. Rodrigues[1], *C.C. C. R. de Carvalho*[1]
[1]*Instituto Superior Técnico,*
Department of Bioengineering- Institute for Bioengineering and Biosciences IBB, Lisboa, Portugal

Backgrounds

Microorganisms colonize most of the surfaces in the World. They grow preferentially attached to surfaces, in a community that confers protection to individuals termed biofilm. The interaction between a bacterial cell and a surface is the first step to the establishment of a biofilm. The process is complex and influenced by the properties of both the surface material and the bacterial cell surface [1]. The local environmental conditions are also quite influential for cell adhesion. Establishment of biofilms in industrial and medical settings cause heat and mass transfer problems and are a source of infections.

Objectives

To understand how surface properties of *Rhodococcus erythropolis* cells change during adhesion and biofilm formation

Methods

Cells response to surface type and environmental conditions was studied by promoting biofilm growth both on metallic and non-metallic surfaces and by using a recirculation system with different types of tubes. Flow cells allowed the access to the different layers of cells in a biofilm.

Conclusions

A poor nutrient medium promoted more biofilm formation than a rich medium, while causing changes in biofilm architecture. The recirculation system showed that the biofilm biomass correlated with the oxygen permeability of the tubes but not to the hydrophobicity of the materials. The cells strongly attached to the surface of the flow cells have less negative net surface charge and a higher saturation degree of their membrane lipids than cells lightly attached. These studies could contribute to new insights into the complex process of cell adhesion and biofilm formation.

[1] Rodrigues and de Carvalho (2015) FEMS Microbiol Ecol 91: fiv135

FEMS7-2145
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHARACTERIZATION AND COMPARATIVE GENOMICS OF A NEW HYDROCARBON-DEGRADING PSEUDOMONAS SP. FROM GULF OF MEXICO

J.L. Rodríguez Mejía[1], L.F. Muriel-Millán[1], E.E. Godoy Lozano[1], S.R. Sánchez Catalán[1], D.X. Sahonero Canavesi[1], W.I. Escobedo Hinojosa[1], L.P. Segovia Forcella[1], R.M. Gutiérrez Ríos[1], M.D.R. Trejo Hernández[2], F.A. Sánchez Flores[1], L. Pardo López[1]
[1]Biotechnology Institute - UNAM, Gulf of México Research Consortium CIGoM, Cuernavaca- Morelos, Mexico
[2]Centro de Investigación en Biotecnología, Gulf of México Research Consortium CIGoM, Cuernavaca- Morelos, Mexico

Backgrounds

In last few years the characterization of new bio-tools for biotechnology applications is increased, the principal activity for this is the seeking of novel microorganisms, these discoveries are couple with the description of new ecosystems. The fusion of these strategies represents a new interdisciplinary approach that improves the way to obtain a specific tool for a specific application.

Objectives

Our aim is discovery bio-tools that have a natural evolve in the degradation of hydrocarbons, principally, bacteria with a wild adaptation for different abiotic conditions and the pollution by xenobiotic compounds.

Methods

The natural hydrocarbon emanation in the Gulf of Mexico and the oil industry activity produce a potential ecosystem for the development of microorganisms with skills to use oil as a unique carbon source. A microbiology approach let us isolated a new *Pseudomonas sp.* from 1000 meters of depth in a water column, able to growth in a mineral media with crude oil as a unique source of carbon.

Conclusions

The complete sequencing of its genome let us to know that this organism is a new specie with close relatives as *Pseudomonas alcaligenes*, and in add a wide comparative bioinformatics analysis describes a conserved set of tools for alkane oxidation and also aromatics hydrocarbons. The genes of this cluster were previously described in other members of Pseudomonas exposing a level of adaptation to the presence of aliphatic hydrocarbons. Growth experiments with specific aliphatic compounds show a taste for short chain alkanes. The results obtained allow us to have a potential hydrocarbon degrading bio-tool.

FEMS7-2695
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DETECTION OF BLAKPC CARBAPENEMASE GENE IN GRAM-NEGATIVE BACTERIA ISOLATED FROM WASTEWATER TREATMENT PLANT (WWTP) IN MEDELLIN, COLOMBIA

E.A. Rodríguez[1], A. Aristizábal[2], L. Arias[3], A. Yamilet[3], J.N. Jiménez[2]
[1]Bacterial Molecular Epidemiology Line. Molecular Microbiology Research Group and Applied & Basic Microbiology Research Group MICROBA, Universidad de Antioquia, Medellin, Colombia
[2]Bacterial Molecular Epidemiology Line. Applied & Basic Microbiology Research Group MICROBA, Universidad de Antioquia, Medellín, Colombia
[3]Microbial Bioprocesses Research group-BIOMICRO, Universidad de Antioquia, Medellín, Colombia

Backgrounds

In Colombia, the problem of resistant to carbapenems Gram-negative bacteria has been evidenced in hospitals but knowledge in wastewater treatment plant (WWTP) is limited.

Objectives

The aim of this study was to determine the presence of Carbapenem-resistant gram-negative bacteria in one WWTP of Medellín, Colombia

Methods

A cross-sectional study was conducted in one WWTP. Four samples of water of affluent and effluent were collected in September 2016. Isolation of bacteria was performed on chromID® CARBA medium. Identification and susceptibility testing were performed using the VITEK®-2 system. Molecular analyzes included PCR for detection of *bla*KPC, *bla*NDM, and *bla*OXA-48 genes.

Conclusions

A total of 44 bacteria were isolated (18 affluent and 26 effluent). Of these, 14 (32%) isolates were resistant to carbapenem and their resistance pattern showed resistances to three or more of the tested antibiotics. *Klebsiella pneumoniae* was high prevalence among carbapenem-resistant isolated species (35.7%, n=5) followed by *Escherichia coli* (14.3%, n=2), *Enterobacter cloacae* (14.3%, n=2), *Citrobacter freundi* (14.3%, n=2) and less frequently (7.1% n=1) *Klebsiella oxytoca, Pseudomonas aeruginosa y Enterobacter aerogenes.* The carbapenemese KPC was detected in all isolates resistant to carbapenem, 9 in the affluent and 5 in the effluent, no other carbapenemases were detected.

This study describes the first detection of KPC-producing Gram negative bacteria in the affluent and effluent of WWTP in Colombia. These results have serious implications for public health because to shows the capacity of this resistance mechanism to persist in the effluent of WWTP and the possibility of dissemination and transferred from the aquatic environment to the human.

FEMS7-0683
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BEHAVIOUR OF ENTERIC BACTERIA AND VIRUSES IN CLAY AND SANDY SOILS AFTER BIOFERTILIZATION WITH SWINE DIGESTATE

D. Rodriguez-Lazaro[1], *G. Fongaro*[2], *M.C. García González*[3], *A. Kunz*[4], *C.R.M. Barardi*[2], *M. Hernandez*[5]
[1]*University of Burgos, Biotechnology and Food Science, 9001, Spain*
[2]*Universidade Federal de Santa Catarina,*
Departamento de Microbiologia- Imunologia e Parasitologia, Florianópolis, Brazil
[3]*ITACyL, BY products Unit, Valladolid, Spain*
[4]*EMBRAPA, Suínos e Aves, Concórdia, Brazil*
[5]*ITACyL, Laboratory of Molecular Biology and Microbiology, Valladolid, Spain*

Backgrounds

Swine digestate can contain high levels of enteric pathogens that may contaminate soil, water and foods.

Objectives

We aimed to evaluate the survival and percolation of several major enteric micoroganisms and to (select the most suitable enteric microorganisms to serve as biomarkers of percolation and leaching after biofertilization with swine digestate.

Methods

We evaluated the survival, percolation and leaching of model enteric pathogens in clay and sandy soils after biofertilization with swine digestate: PhiX-174, mengovirus (vMC_0), *S*. Typhimurium and *Escherichia coli* O157:H7 were used as biomarkers.

Conclusions

The survival of viruses in clay soil was significantly lower than in sandy soil ($i T_{90}$ values of 10.520 ± 0.600 *vs* 21.270 ±1.100 and 12.040 ± 0.010 *vs* 43.470 ± 1.300, respectively) and PhiX-174 showed faster percolation and leaching in sandy soil than clay soil ($i T_{90}$ values of 0.46 and 2.43, respectively). *S*. Typhimurium was percolated and inactivated more slowly than *E. coli* O157:H7 ($i T_{90}$ values of 9.340 ± 0.200 *vs* 6.620 ± 0.500 and 11.900 ± 0.900 *vs* 10.750 ± 0.900 in clay and sandy soils, respectively), such that *E. coli* O157:H7 was transferred more quickly to the deeper layers of both soils evaluated (percolation). Consequently, *E. coli* O157:H7 may serve as a useful microbial biomarker of depth contamination and leaching in clay and sandy soil and that bacteriophage could be used as an indicator of enteric pathogen persistence. Our study contributes to development of predictive models for enteric pathogen behaviour in soils, and for potential water and food contamination associated with biofertilization.

FEMS7-2500
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHARACTERISING THE CULTURABLE BACTERIA FROM MINKE WHALE (BALAENOPTERA ACUTOROSTRATA) GUT BY 16S RRNA AMPLICON SEQUENCING

I.S. Roiha[1], C.S. Svanevik[1], B.T. Lunestad[1]
[1]National Institute of Nutrition and Seafood Research, Seafood safety and health, Bergen, Norway

Backgrounds

Whales are homeothermic animals in an environment dominated by heterothermic ones, and as such represent an exclusive reservoir of microbes. Investigating the cultured portion of bacteria from the gut microflora gives new insight to the diversity and functional aspects of the gut microbial communities, including nutritional functions, biogeochemical cycling, and of possible antibiotic resistance.

Objectives

In this study, we explored the gut microbiomes of jejunum, ileum, and colon samples from four minke whale (*Balaenoptera acutorostrata*) individuals, by traditional microbiological methods and molecular analyses. For increased understanding of the taxonomic and functional diversity of the whale gut microbiome, 16S rRNA amplicon metagenomic sequencing were applied to investigate the culturable portion of bacteria from the gut.

Methods

Gut samples from whales, caught in the Barents Sea, were obtained from a commercial Norwegian whaling vessel. Aerobic and anaerobic plate counts were set up, parameters included total plate count (TPC), Enterobacteriaceae, Enteroocci, *Listeria monocytogenes*, *Salmonella*, pathogenic vibrios, *Lactobacillus* sp., and *Pseudomonas* sp. Bacterial colonies were harvested from the agar plates in bulks for future DNA analysis. Glycerol stocks were made for future microbiological investigations.

Conclusions

Parameters included analyses of *Listeria*, *Salmonella,* and pathogenic vibrios, neither of which were detected in the gut content. Higher bacterial counts were found in the jejunum and ileum samples compared to colon samples. Results from the 16S rRNA amplicon sequencing will be presented.

FEMS7-2522
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHARACTERISING THE GUT MICROBIOTA OF MINKE WHALE (BALAENOPTERA ACUTOROSTRATA) – MEMBERS AND FUNCTIONS

I.S. Roiha[1], B. Wilson[2], P. Whatmore[3], B.T. Lunestad[1]
[1]National Institute of Nutrition and Seafood Research, Seafood safety and health, Bergen, Norway
[2]University of Bergen, Department of Biology, Bergen, Norway
[3]National Institute of Nutrition and Seafood Research, Fish Nutrition, Bergen, Norway

Backgrounds

Whales are homeothermic animals in an environment dominated by heterothermic ones, and as such represent an exclusive reservoir of microbes.

Objectives

In this study, we explored the taxonomic and functional diversity of gut microbiomes from four minke whale (*Balaenoptera acutorostrata*) individuals, by shotgun metagenomic sequencing of jejunum, ileum, and colon samples.

Methods

Gut samples from whales, caught in the Barents Sea, were obtained from a commercial Norwegian whaling vessel, and DNA extracted and sequenced by the Illumina HiSeq 2500 platform.

Conclusions

All of the three domains Archaea, Bacteria, and Eukaryota were well represented in the jejunum and ileum samples; however, the Archaea were noticeably absent from the colon. Whilst gut microbiomes were dominated by the Bacteria (comprising 32-97 % relative abundance), ileum samples from two of the whales were dominated (61-63 % relative abundance) by the Eukaryota. The highest relative proportions of Bacteria (96-97 %) were found in the colon. In the Bacterial fractions, higher diversities were seen in the jejunum and ileum samples compared with those of the colon.

Investigations into the taxonomic diversity continue, to compare the gut microbiome of the four different whale individuals (consisting of three females [one young and two mature] and one mature male). Microbial genomes have been assembled from the metagenomic data and the functional role of these uncultured organisms in the whale gut is being investigated. These data will be presented.

FEMS7-0253
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL SELECTION AND STOICHIOMETRY OF GLUCOSE AND XYLOSE FERMENTATION IN MIXED CULTURE ECOSYSTEMS OF ANAEROBIC SEQUENCING BATCH REACTORS

J.L. Rombouts[1], D.G. Weissbrodt[1], R. Kleerebezem[1], M.C.M. van Loosdrecht[1]
[1]Delft University of Technology, Biotechnology, Delft, The Netherlands

Backgrounds

Enrichment culture experiments can elucidate mechanisms behind microbial selection and diversity in mixed-culture fermentation systems. Here, microbial selection and conversion stoichiometries were studied during the fermentation of glucose and xylose, which are two monomers abundant in agro-waste streams. Anaerobic sequencing batch reactors (ASBRs) were used for enrichment of the strain with highest growth rate on the respective substrate. Do both enrichments result in one dominant organism? And do xylose or glucose lead to a similar product spectrum in chemical steady state?

Objectives

1. Compare the metabolic stoichiometry for glucose and xylose fermentation at steady state
2. Analyze the community structure to evaluate the hypothesis

Methods

Two 2-L ASBRs were operated in 6h cycles at 30±0.5 °C and pH 8.0±0.1, with a short hydraulic residence time of 8h, after inoculation with bovine rumen fluid. A medium composed of inorganic salts (Temudo *et al.* 2007) was fed with either glucose or xylose as sole carbon sources at 4 g/L. Main products were quantified by HPLC and off-gas analysis. The stoichiometries of the metabolisms were verified with COD balancing. Community compositions of the suspensions were analyzed by light microscopy, PCR–DGGE and band sequencing on the V3-16S rRNA gene pool, and 16S rRNA-targeted FISH.

Conclusions

1. A similar product spectrum dominated by coupled acetate/ethanol formation was obtained for both substrates, reported earlier (Temudo *et al.* 2007).
2. For glucose, one *Enterobacter* population dominated.
3. For xylose, one *Citrobacter* population dominated, additional to a *Clostridium* population
4. FISH confirmed dominance of Gammaproteobacteria for both enrichments

Following up, we will characterize a mixed substrate ASBR, hypothesizing two organisms will dominate.

FEMS7-0962
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RIVER BIOFILM RESPONSES TO MULTIPLE STRESSORS: EFFECTS OF A CONCENTRATION GRADIENT OF WASTEWATER EFFLUENTS ARE MODULATED BY DESICCATION

F. Romero[1], *S. Sabater*[1], *V. Acuña*[1]
[1]*Catalan Institute for Water Research, Recursos i Ecosistemes, Girona, Spain*

Backgrounds

Wastewater treatment plants (WWTPs) release large amounts of chemical compounds such as inorganic micropollutants and nutrients to river and stream ecosystems. Discharge from WWTPs results in high concentrations of chemicals immediately after the discharge point, whereas downstream sites usually show smaller concentrations due to natural dilution and attenuation of chemicals. Microorganisms in biofilms exposed to these chemicals play a major role on ecosystem maintenance and functioning. Further, climate change-related stressors also affect the biological communities. In particular, flow interruption and desiccation affect systems under water scarcity, and exert formidable effects on microbial communities.

Objectives

Here we aim at evaluating the response of river biofilms across a concentration gradient of a complex chemical stressor (i.e. WWTP effluent) within the context of a climate-change-related stressor (i.e. desiccation).

Methods

To that purpose, we used a mesocosms approach to expose river biofilms affected or not by desiccation to a concentration gradient of WWTP effluents using a regressional design. The response of the biofilm community was studied at two levels: (i) bacterial diversity and composition was assessed by means of high-throughput sequencing of 16S rRNA genes, and (ii) community functioning was assessed by quantitative PCR to determine the prevalence of genes involved in major ecosystem functions such as nitrogen metabolism and photosynthesis.

Conclusions

We hypothesize that desiccation will significantly modify the community responses to the pollution gradient and this will be reflected in the biofilm functioning.

FEMS7-3221
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIOGEOCHEMICAL CHARACTERIZATION OF THE SEDIMENT OF THE SEINE RIVER ESTUARY

C. Roose-Amsaleg[1], *A. Laverman*[2], *A. Thibault*[1], *A. Huguet*[1]
[1]*Metis, University Pierre and Marie Curie, Paris, France*
[2]*Ecobio, University Rennes 1, Rennes, France*

Backgrounds

At the land – sea interface, estuaries receive dissolved and suspended matter from both terrestrial and ocean environments. The transport and fate of this matter (organic carbon, nutrients and pollutants) depends on biogeochemical cycling within the water column and sediment. The Seine river estuary (France), under high anthropogenic influence, presents important nitrogen and organic carbon loads.

Objectives

Our **objective** was to determine benthic nitrate reduction and carbon oxidation rates, enumerate nitrogen transforming bacteria in relation to carbon quantity and quality in the Seine Estuary, France.

Methods

To this end, surface sedimentary nitrate reduction and ammonium production rates from 4 sites along the Seine estuary were determined. The microbial presence was estimated by qPCR of functional genes. Furthermore the organic matter was quantified and qualified (total C and N, sugar and protein concentrations, solid state ^{13}C NMR).

Conclusions

Sedimentary OM characterization showed the variation of OM sources (allochthonous vs. autochthonous) along the estuary. Denitrifiers represented the most abundant nitrogen cycle microorganisms (14-22% of the total microbial community) in April 2016. In addition, a small fraction of the bacteria able to mediate Dissimilative Nitrate Reduction to Ammonium was detected (0.4-0.79%). These proportions were consistent with potential nitrate reduction and ammonium production rates.

FEMS7-2049
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MINING FOR DETOXIFICATION GENES IN THE GUT MICROBIOTA OF A MONOPHAGOUS INSECT

T. Rosas Pérez[1], *F. González Serrano*[1], *J. Marín Miret*[1], *A. Moya*[1]
[1]*Institute for Integrative Systems Biology, Evolutionary Genetics, Valencia, Spain*

Backgrounds

Brithys crini (Lepidoptera:Noctuidae) is a monophagous insect which feeds exclusively on leaves of *Pancratium maritimum,* a plant that grows on coastal sand dunes of the Mediterranean region. Several Pancratium species contain different alkaloids in large amounts that have toxic effects and have a role in protecting the plant against natural enemies.
Specialist insects like *B. crini* must have a gut microbiota capable of surviving to the toxicity of its diet or/and of catabolizing or transforming the toxic compounds.

Objectives

To investigate the capabilities of the *B. crini* gut microbiota to cope with the alkaloids on its diet, midgut and hindgut of 20 larvae were dissected and the purified DNA from the sections was sequenced.

Methods

The metagenome of the gut microbiota was sequenced using the Illumina MySeq technology. Reads and contigs were annotated and genes related to detoxification, aromatic compounds catabolism, metabolism of secondary metabolites and defense mechanisms where mined. Taxonomic and functional classifications were compared between midgut and hindgut datasets.

Conclusions

The classified sequences derived from bacteria belong to 27 different phyla. The most abundant genera were Bacteroides, Clostridium, Parabacteroides, Desulfovibrio, Enterococcus, Enterobacter, Klebsiella, Escherichia and Mycobacterium, but their proportions varied between midgut and hindgut. Also a higher bacerial diversity was found in the hindgut section.
We found that while some genera have many genes related to catabolism of secondary metabolites, most of the bacterial groups may relay on a variaty of efflux pumps and transporters to cope with the toxic compounds in the gut.

FEMS7-0636
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

POULTRY-RELATED MICROBIOME CHANGES FROM EMBRYONIC DEVELOPMENT TO FINAL PRODUCT: A SINGLE PASTURED-RAISED FLOCK FARM-TO-FORK ANALYSIS

M.J. Rothrock - Jr.[1], *A. Locatelli*[1], *K. Hiett*[1], *A. Caudill*[2]
[1]*USDA-ARS, US National Poultry Research Center, Athens, USA*
[2]*University of Georgia, Department of Public Health, Athens, USA*

Backgrounds

While conventionally grown poultry continues to dominate the U.S. poultry industry, there is an increasing demand for locally-grown, “all natural” alternatives. Unfortunately, limited research has been done on this type of poultry management practice, and thus many of these management effects on the environment, poultry products, and human health is unknown.

Objectives

The use of next generation sequencing allows for not only the gross (e.g. community structure) but also fine-scale (e.g. genera abundances) examination of complex microbial communities. Utilizing these technologies can provide a better understanding of the poultry microbiome and how it changes throughout a flock’s life cycle to better elucidate not only the overall microbial ecology of these communities, but specifically the ecology of the foodborne pathogens inherent within poultry production.

Methods

Broiler samples were taken during the entire flock life cycle, including Hatchery, Brood, Pasture, Processing, and Final Product, as well as samples from other farm animals in close contact with the broilers. Genomic DNA was extracted, 16S microbiomic profiles were generated (Illumina MiSeq), and microbiomes were analyzed and compared using QIIME 1.91 to determine how microbiomes shifted throughout production continuum, as well as what environmental or management factors may be influencing these shifts.

Conclusions

Significant microbiome shifts occurred during the life cycle of this broiler flock, with microbiomes clustering based on sample type and stage of production continuum. Additionally, while this flock was considered “free-range” with access to and contact with other farm animals, the poultry fecal microbiomes remained distinct from the non-poultry animals throughout their lives.

FEMS7-3090
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

METAGENOME AND ACTIVE MICROBIOME PROFILING IN AMMONIA-RICH ANAEROBIC DIGESTION

J. Ruiz Sánchez[1], S. Campanaro[2], M. Guivernau[1], B. Fernandez[1], F.X. Prenafeta-Boldú[1]
[1]IRTA, GIRO, Caldes de Montbui, Spain
[2]Universita di Padova, Biology, Padova, Italy

Backgrounds

Ammonia released during the anaerobic digestion (AD) of N-rich organic wastes is a well-known inhibitor of methanogens and, particularly, the acetoclastic archaea. Recently, an alternative pathway known as syntrophic acetate oxidation (SAO) has been identified as possible strategy to overcome this inhibition.

Objectives

This study is aimed at gaining a better understanding of the microbial community interactions in industrial AD reactors operated at different total nitrogen ammonia (TAN) levels.

Methods

A shotgun metagenomics sequencing and binning of scaffolds into population genomes has been performed with the biomass from four full-scale digesters (R1, R2, R3 and R4) operated under 6, 5, 2 and 2 g_{TAN} L^{-1}.Total DNA and RNA from the biomass of each digester was extracted. Genomic DNA was prepared for paired-end sequencing using Nextera Kit and cDNA obtained obtainedwasas used for high throughput sequencing purposes, utilizing equipment.

Conclusions

The combination of DNA and RNA-based taxonomic analysis and functional genetic studies allowed the reconstruction of Wood–Ljungdahl (W-L) pathway in *Bacteroidetes* and *Chloroflexi* genomes bin. This fact evidenced that both phyla might harbour SAOB potential and were in agreement with high abundance and metabolic activity in digesters exposed to high TAN levels. W-L pathway is fundamental for acetate conversion to H_2 and CO_2, which are the principal substrate for hydrogenotrophic methanogens that were found out the most active archaeal community in TAN-rich digesters. Consequently, the obtained results point to the occurrence of novel, to our knowledge not yet described, syntrophic associations between SAOB-HM.

FEMS7-3095
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL COMMUNITY DYNAMICS IN NITROGEN RICH DIGESTERS: IMPACT OF EFFLUENT RECIRCULATION

J. Ruiz Sánchez[1], M. Romero[1], S. Astals-Garcia[2], B. Fernandez[1], F.X. Prenafeta-Boldú[1]
[1]IRTA, GIRO, Caldes de Montbui, Spain
[2]University of Queensland, Advanced Water Management Centre - AMWC, Queensland, Australia

Backgrounds

Anaerobic digestion (AD) is a widely consolidated biotechnology for the valorization of organic wastes.

Objectives

The AD microbiome response towards increasing ammonia concentrations, up to inhibitory levels (<6 $gN\text{-}NH_4^+\ L^{-1}$) was studied in two lab-scale mesophilic AD bioreactors operated with (AD1) and without (AD2) recirculation.

Methods

The active microbial community from the biomass of each digester was characterized by triplicate and at different operational stages by extracting the total RNA and generating cDNA, libraries. Total transcripts of ribosomal 16S rRNA and mcrA genes were determined by qPCR. Meanwhile, the same cDNA was used for high throughput sequencing purposes.

Conclusions

No significant differences were observed between the two digesters, concerning physic-chemical and efficiency parameters, but a drastic microbial community shift was observed between both reactors, as evidenced by biodiversity indexes and multivariate analysis on the species abundance matrix. Active biomass in AD1 was dominated by *Pseudomonas* spp., possibly due to their degradation capability against polymeric materials (lingocelullose) that accumulated due to recirculation. Meanwhile, the phyla *Bacteroidetes* and *Firmicutes* were the predominant population in AD2. Putative acetogenic bacteria within these groups have recently been described as syntrophic acetate oxidation bacteria (SAOB). Despite differences in the bacterial communities, hydrogenotrophic methanogens (HM) belonging to the genera *Methanobacterium* and *Methanoculleus* were the prevalent archaeal counterparts in both bioreactors. This finding highlights the enrichment of HM, which tend to be relatively tolerant towards ammonia, and the establishment of potential syntrophic interactions with exoelectrogenic *Pseudomonas* spp., which might in fact be able to carry out the SAO pathway.

FEMS7-3142
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SHARING OF BACTERIAL STRAINS BETWEEN MATERNAL PRECOLOSTRUM AND INFANT SALIVA: CULTURE INDEPENDENT AND CULTURE DEPENDENT APPROACHES

L. Ruiz[1], C. García-Carral[2], C. Beatriz[3], A. Checa[4], J.M. Rodriguez[5]
[1]IPLA-CSIC, Department of Microbiology and Biochemistry of Dairy Products, Villaviciosa-Asturias, Spain
[2]Universidad Complutnse de Madrid,
Departamento de Nutrición- Bromatología y Tecnología de los Alimentos, Madrid, Spain
[3]Universidad Complutense de Madrid,
Departamento de Nutrición- Bromatología y Tecnología de los Alimentos, Madrid, Spain
[4]Centro de Salud Arrabal, Centro de Salud Arrabal, Zaragoza, Spain
[5]Universad Complutense de Madrid,
Departamento de Nutrición- Bromatología y Tecnología de los Alimentos, Madrid, Spain

Backgrounds

The infant oral microbiome exerts an important role in the establishment of the infant gut microbiome since it contains the first commensals that will reach and stably colonize the infant gut, but it also influences the dental health of the adult. In this context, human milk represents a source of bacteria for the initial establishment of the oral (and gut) microbiomes in the breastfed infant although little is known on the specific mechanisms that condition the early establishment and development of the infant oral microbiomes.

Objectives

To investigate the maternal colostrum and infant oral microbiomes to identify bacterial species (strains) shared in the maternal-infant dyad.

Methods

Maternal pre-colostrum and infant saliva were collected from 19 mother-infant pairs and their microbiomes were analyzed through 16S rRNA metagenomics sequencing. Bacteria were also cultured, isolated and identified by MALDI-TOF. RAPD-PCR was performed on those isolates that, belonging to the same species, were shared in the maternal and infant samples of the same pair.

Conclusions

Maternal pre-colostrum and infant salivary microbiomes were significantly different from each other, although certain groups were consistently shared between both sample types. *Streptococcus* and *Staphylococcus* spp were the most abundant groups in all samples. In 13 pairs, at least one isolate from the same species was recovered from the colostrum and the salivary sample. From those, the isolates obtained from colostrum and infant saliva in 8 of the pairs could not be distinguished by RAPD-PCR profiling, thus suggesting the sharing of bacteria at strain level between milk and infant oral microbiomes.

FEMS7-2131
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CLOSTRIDIUM DIFFICILE COLONIZATION AND GUT MICROBIOTA IN HOSPITALIZED IBD PATIENTS

A. Mahnic[1], S. Pintar[2], A. Kocuvan[2], P. Skok[3], M. Rupnik[4]
[1]National Laboratory of Health Environment and Food, Department for microbiological research, Maribor, Slovenia
[2]University of Maribor- Faculty of Medicine, Department for Microbiology, Maribor, Slovenia
[3]University Medical Centre Maribor, Department of gastroenterology, Maribor, Slovenia
[4]National Laboratory of Health Environment and Food and University of Maribor faculty of Medicine, Department for microbiological research, Maribor, Slovenia

Backgrounds

Gut microbiota performs numerous essential metabolic, developmental and immune functions and defends against invading pathogens and resident opportunists. Imbalance in gut microbiota can destabilize homeostatic relationship and lead to various health issues. Studies show strong association between dysbiotic gut microbiota and several gastrointestinal diseases such as inflammatory bowel disease (IBD), irritable bowel syndrome and *Clostridium difficile* infection (CDI).

Objectives

Aim of this study was to analyze *C. difficile* colonization and gut microbiota of patients hospitalized in the Department of gastroenterology (UMC Maribor, Slovenia) including IBD and non IBD patients compared to a group of healthy individuals.

Methods

Study includes a group of 94 hospitalized patients and a control group of 55 healthy volunteers. *Clostridium difficile* colonization was examined with specific real-time PCR. 16S metagenomes were acquired by paired-end sequencing on Illumina MiSeq platform targeting V3-V4 hypervariable region of the bacterial 16S rRNA gene.

Conclusions

Rate of *C. difficile* colonization is similar between IBD and non IBD patients (20.4 % and 25.0 %, respectively) while significantly lower among healthy volunteers (1.8 %).

Microbiome data can differentiate hospitalized patients from healthy controls. Dissimilarities are evident as lower alpha diversity (Shannon $p<0.001$) as well as differences in community structure (AMOVA $p<0.001$). Focusing solely on hospitalized patients we were able to show that IBD patients have a lower alpha diversity (Shannon $p=0.008$) and distinguish from other patients based on bacterial community (AMOVA $p=0.014$) with the most prominent being decreased abundance of genus *Akkermansia.* No significant differences were found between *C. difficile* colonized versus non-colonized patients.

FEMS7-2491
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE CHARACTERISTICS OF CANDIDA ALBICANS "GROWTH FITNESS" UNDER THE NEW SYNTHETIC PORPHYRIN INFLUENCE

M. Rusakova[1], *O. Ruschak*[2], *S. Vodzinskiy*[1], *Y. Ishkov*[1]
[1]*Odessa I. I. Mechnikov National University, Biotechnological Scientific Education Center, Odessa, Ukraine*
[2]*Odessa I. I. Mechnikov National University, Microbiology- Virology and Biotechnology Department, Odessa, Ukraine*

Backgrounds

In vivo Candida albicans is polymorphic microorganism. It is detected that *C. albicans* hyphae have greater invasive potential than the yeast forms. In this existence form *C. albicans* is more resistant to antifungal substances.

There have been several reports on the use of porphyrins to kill *C. albicans*. However, there has been much less study on the types of physicochemical properties necessary in a molecule in order to make it effective in microbial killing.

Objectives

The aim was to determine the sensitivity of different *Candida albicans* morphological forms to a number of synthetic porphyrins.

Methods

During microorganism cultivation in the Sabouraud and Spider media there were determined differences between *C. albicans* forms. In the first medium the most cells had an oval shape, were actively dividing and eventually generated a continuous biofilm. In the case of *C. albicans* growth in Spider medium cells elongated, were not practically separating from each other during the division that resulted to the formation of pseudohyphae.

Conclusions

Results of experimental investigations have demonstrated that different *C. albicans* forms can be effectively killed by the porphyrins. *C. albicans* biofilm cells are more resistant to low concentrations of the compounds however the significant inhibition of the microbial community is in the presence of 1 μM. Modification of porphyrin molecules by N-methyl-6-quinolinyl increases the fungicidal effect on the yeast cells, in contrast to N-methyl-7-quinolinyl derivatives that are effective against hyphal structures.

So, therapy with porphyrins has the potential to evolve into a useful treatment for difficult to eradicate *C. albicans* infections.

FEMS7-3022
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIODEGRADATION OF ORGANOPOLLUTANTS BY SOIL MICROORGANISMS ISOLATED FROM POLLUTED SITES IN CHINA

Z. Rybková[1], K. Malachová[1], J. Červeň[1], R. Ji[2]
[1]University of Ostrava, Department of Biology and Ecology- Faculty of Science, Ostrava, Czech Republic
[2]Nanjing University,
State Key Laboratory of Pollution Control and Resource Reuse- School of the Environment, Nanjing, China

Backgrounds

Soil samples were collected from five agriculturally and industrially contaminated locations in China. These sites included a pesticide production plant, fields heavily treated with pesticides, and soil polluted with different organic pollutants and heavy metals.

Objectives

Aim of this work was to isolate bacterial and fungal strains with a potential for biodegradation of recalcitrant organopollutants.

Methods

Bacterial and fungal strains were isolated using low-nutrient-concentration media with Bisphenol A (BPA), Tetra-Bromo Bisphenol A (TBBPA), Sulfamethoxazole (SMX) and Diclofenac (DF) as single carbon sources. Experiments were conducted with selected isolates using ^{14}C labeled BPA and TBBPA. The biodegradation efficiency was evaluated using HPLC-LSC and the degradation products were identified. Biodegradation potential of four isolated strains of microorganisms was also checked by degradation of recalcitrant synthetic dyes.

Conclusions

A total of 151 bacterial and 41 fungal strains were isolated from the soil samples using diclofenac, sulfamethoxazole, BPA, and TBBPA as single carbon sources. Experiments with ^{14}C labelled BPA showed that the fungal isolate ST19 had a higher ability to mineralize BPA than the bacterial strain 12B4. In the study, the ability of the above strains to degrade synthetic dyes was also documented.

Acknowledgements: The study was supported by the projects: Podpora bilaterální spolupráce v oblasti VŠ vzdělávání, vědy a výzkumu mezi univerzitami Moravskoslezského kraje a čínskými univerzitami a výzkumnými organizacemi (reg. no. 00955/2015/RRC) and TEWEP LO1208 of the National Feasibility Programme I of the Czech Republic.

FEMS7-3267
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EVALUATION OF THE ANTIBACTERIAL POTENTIAL OF SOME ESSENTIAL OILS IN BIOLOGICAL CONTROL AGAINST PHYTOPATHOGENIC AGENT PSEUDOMONAS SYRINGAE PV. TOMATO DC3000 RESPONSIBLE FOR THE TOMATOES SPECK

A. SABIR[1], B. El Khalfi[1], F. Errachidi[1], A. Serrano Delgado[2], A. Soukri[1]
[1]Faculty of Sciences, Biology, Casablanca, Morocco
[2]Instituto de Bioquímica Vegetal y Fotosíntesis CSIC-
Universidad de Sevilla- Centro de Investigaciones Científicas Isla de la Cartuja- Américo Vespucio 49 - 41092-Sevilla- Spain, Biology, Sevilla, Spain

Backgrounds

The essential oils are exploited in various fields due to their many therapeutic virtues. In phytotherapy, they can present a serious candidate to replace the various chemical pesticides commonly used against infectious diseases of fungal and bacterial origin.

Objectives

The aim of this study is to determine the chemical composition and the antimicrobial activity of six essential oils against the strain *Pseudomonas syringae pv.tomato DC3000* that is responsible for bacterial speck and resistant to rifampicin, in order to develop a biological control means.

Methods

Essential oil from the plant collected in north centre region of Morocco obtained by hydro-distillation were analyzed by gas chromatography equipped with flame ionisation detector (GC-FID).The antimicrobial activity of the oils was determined using well diffusion, micro atmosphere, and determination of MIC/CMB methods. An acp analysis was also done.

Conclusions

The GC analysis showed that the major constituents of the oils were monoterpene hydrocarbons and phenolic monoterpenes, but the concentration of these compounds varied greatly among the oils examined. The results of the antimicrobial assay showed that four essential oils inhibited the growth of *Pseudomonas syringae pv.tomato DC3000* namely *Thymus vulgaris*, *Citrus limonum*, *Mintha puligium* and *Eucalyptus globulus.* The best bacteriostatic and bactericidal effect remains that of *Eucalyptus globulus*. The results of this study confirmed the possibility of using these oils or some of their components in agriculture to prevent the growth of this phytopathogen bacterium and extend the shelf-life of tomatoes.

FEMS7-2298
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ACTINOBACTERIA RESPIRATION AT SITES DIFFERING IN HEAVY METAL CONTAMINATION WAS ALTERED BY SUPPLEMENTATION WITH ORGANIC SUBSTRATE.

M. Sagova-Mareckova[1], *P. Madrova*[1], *J. Kopecky*[1]
[1]*Crop Research Institute, Epidemiology and ecology of microorganisms, Prague 6, Czech Republic*

Backgrounds

At sites contaminated with heavy metals, the composition and activity of microbial communities are influenced by stress factors one of which is the content and character of organic matter. *Actinobacteria* contribute to breakdown of organic matter and survive in heavy metal contaminated conditions because they possess efflux resistances enabling cell detoxification.

Objectives

The aim of the study was to show how organic matter decomposition is affected by heavy metal contamination in *Actinobacteria*.

Methods

We selected two sites differing in long-term contamination by cadmium (L low, H high contamination). We assessed the changes in respiration after addition of cadmium and two organic substrates, cellobiose and straw using OxiTop system. We assessed quantity of actinobacteria by qPCR, diversity of cellobiohydrolase by specific primers, and bacterial diversity by Illumina sequencing.

Conclusions

The addition of cadmium did not have any effect on respiration but in the cellobiose-cadmium treatment. The addition of organic substrate did not influence the quantity of actinobacteria but in the cadmium-cellobiose treatment at H site. Sequences of cbh gene (coding for cellobiohydrolase) differed significantly (Libshuff) between the sites H and L, both untreated and in the straw-cadmium threatment. The two sites differed in actinobacteria community composition after addition of straw, *Micrococcaceae* increased at both sites, *Nocardiaceae* and *Cellulomonadaceae* increased at H site, and *Thermomonosporaceae* increased at L site. In conclusion, the microbial communities at site with low contamination seemed to be more resilient being less affected after additions of organic substrates.

FEMS7-3231
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BUTYRATE PRODUCTION IN FUSOBACTERIUM NUCLEATUM IS ENHANCED BY CROSS-FEEDING WITH STREPTOCOCCUS GORDONII

A. Sakanaka[1], M. Kuboniwa[1], A. Amano[1]
[1]Osaka University Graduate School of Dentistry, Preventive Dentistry, Suita- Osaka, Japan

Backgrounds

Streptococcus gordonii, an oral commensal, has been considered to be an accessory pathogen that is able to enhance the colonization of periodontal pathogens. We previously showed that excretion of ornithine by *S. gordonii* helped bolster biofilm formation by a periodontitis-associated bacterium, *Fusobacterium nucleatum*.

Objectives

The objective of this study is to further dessect the metabolic interactions between two organisms.

Methods

Co-culture experiments were conducted using transwell setup, where *F. nucleatum* and *S. gordonii* were placed in the lower and upper chambers, respectively. After 6 h of incubation, metabolic profiles of *F. nucleatum* cells and culture supernatants were determined using CE-TOF-MS.

Conclusions

Metabolomics analysis showed significant increases in the extracellular level of ornithine and the intracellular levels of acetylornithine and putrescine in *F. nucleatum* when in co-culture, providing evidence for our previous finding; ornithine cross-feeding. In addition, the presence of *S. gordonii* elevated the intra- and extracellular levels of other amino acids, including alanine and glutamate in *F. nucleatum*, suggesting multiple amino acids cross-feeding. Furthermore, pathway analysis suggested major changes in fatty acid metabolism in *F. nucleatum* co-cultured with *S. gordonii*. Indeed, drastic increase in butyrate level was observed in the supernatants of co-cultures as compared to the respective mono-cultures, indicating enhanced formation of butyrate by *F. nucleatum*, possibly due to activated amino acid fermentation. Considering that enhanced butyrate production involves pathogenesis of periodontitis, our findings highlight the pathogenic potential of *S. gordonii* via cross-feeding with *F. nucleatum*, providing further evidence for the properties of *S. gordonii* as an accessory pathogen.

FEMS7-1410
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EVALUATION OF THE BACTERIAL COMMUNITY TO ASSESS THE TRACEABILITY OF BOTTLED WATER OF DIFFERENT MINERAL WATER BRANDS DURING THE SHELF LIFE

L. Sala-Comorera[1], A. Casanovas-Massana[2], C. García-Aljaro[1], A.R. Blanch[1]
[1]University of Barcelona, Department of Genetics- Microbiology and Statistics, Barcelona, Spain
[2]School of Public Health- Yale University-,
current address: Department of Epidemiology of Microbial Diseases, New Haven, USA

Backgrounds

Natural mineral waters are complex environments containing a high microbial diversity, which is characteristic of each spring. Once bottled, these communities may change over time until the water is consumed. The bottling material is believed to play a major role in the succession of these populations.

Objectives

The microbial community structure and diversity of three natural mineral water brands were investigated over 3 months after bottling in glass and PET bottles for their traceability.

Methods

Each mineral water was bottled on the same day from the same spring in glass and PET. Samples were processed on days 1, 7, 15, 21, 30, 60 and 90 after bottling. Culture-dependent: (heterotrophic plate count) and culture-independent methods: fluorescent microscopy with vital dyes), PCR-DGGE (polymerase chain reaction -denaturing gradient gel electrophoresis) and bacterial 16S rRNA gene amplicon 2x300 bp paired-end sequencing on the MiSeq Illumina platform were used.

Conclusions

Shifts in bacterial composition at different times during the 3 months were observed, however the cluster analysis of the DGGE fingerprints and Illumina sequencing revealed that the samples clustered mainly according to the brand and the bottle material. No difference in the number of total, viable and culturable bacteria counts were observed among mineral water bottled with PET and glass during long term storage. Some of the water brands and/or material had a distinct microbial community structure clearly distinguishable from the others, which suggests the possibility of defining a molecular marker for traceability.

FEMS7-0572
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECTS OF CRUDE OIL ON MICROBIAL DENITRIFICATION AND N2O PRODUCTION IN SALT MARSH SEDIMENTS

H. Ribeiro[1], P. Salgado[1], C.M.R. Almeida[1], A.P. Mucha[1], C. Magalhães[1]
[1]Interdisciplinary Centre of Marine and Environmental Research CIIMAR/CIMAR, EcoBioTec Laboratory, Matosinhos, Portugal

Backgrounds

Denitrification is a key process for the reduction of external N loads and alleviation of eutrophication in coastal waters. Also, denitrification is an important process on the production of nitrous oxide (N_2O), a potent greenhouse gas. Additionally, estuaries receive and tend to accumulate pollutants, such as petroleum hydrocarbons (PHC). Therefore, the evaluation of the impact of PHC on denitrification process and N_2O production is of crucial importance.

Objectives

We assessed the impact of PHC contamination on microbial denitrification, in a five months greenhouse experiment. In addition, the role of *Juncus maritimus*, an estuarine plant, comparatively to non-vegetated sediments was also evaluated.

Methods

Both sediments (vegetated and non-vegetated) were mixed manually with Arabian Light crude oil (supplied by an oil refinery) to a concentration of 5 ml L^{-1} wet sediment. Similar triplicate vegetated and non-vegetated vessels were prepared without crude oil contamination as control. Denitrification and N_2O potential rates were measured, along time, using the acetylene method.

Conclusions

Results revealed that, in both sediments, denitrification rates did not seem to be affected by the addition of crude oil. On the other hand, crude oil clearly enhanced N_2O production in vegetated sediments, but not in non-vegetated sediments. This study provides insights into the potential effects of crude oil on denitrification and N_2O production in estuarine environments, with profound implications for the management of aquatic ecosystems regarding eutrophication and global warming.

FEMS7-1979
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

GENE EXPRESSION ANALYSIS OF SECRETION SYSTEM DURING INTERACTION OF METHYLOBACTERIUM MESOPHILICUM SR 1.6/6 WITH THE HOST PLANT

J.K. Salguero Londoño[1], W.L. Araujo[1], M. Nobrega Dourado[1], D. Souza Santos[1], A. Camargo Neves[1]
[1]Institute of Biomedical Sciences - University of Sao Paulo, Microbiologia, São Paulo, Brazil

Backgrounds

Methylobacterium genus is composed by pink-pigmented facultative methylotrophic bacteria that are able to promote plant growth, increase photosynthetic activity of the host plant and reduce the incidence of pathogens. The *M. mesophilicum* strain SR1.6/6 is a bacterium isolated from citrus and due the interaction with the host plant has been the focus of several studies.

Objectives

This project, evaluated the identification and the expression of some genes related to codification of protein secretion system that could be involved in *M. mesophilicum*-citrus (*Citrus* sinensis) interaction during colonization of this bacterium through the recognition of root exudates compounds released by the host plant. We evaluated the bacterial cells adhered to the roots forming biofilm and bacterial cells in suspension compared to control (bacterial cells without plant).

Methods

For expression analysis, genes that encode protein from secretion system and multidrug efflux pumps were evaluated by qPCR. Then, the *M. mesophilicum* SR1.6/6 genome was reevaluated and genes related to transport systems were reannotated employing bioinformatics tools (BLASTp, BLASTx and JGI), comparing to previously described secretion systems genes. The exudates released by citrus roots, were identified by gas chromatography mass spectrometry (GC-MS)

Conclusions

It was found in the genome of *M. mesophilicum* SR1.6/6 type I, II and V secretion system, multidrug efflux pumps and related ABC transporters. Citrus root exudates released are composed mainly by sugars, organic acids, organic compounds, aminoacids and lipids. Gene expression analysis suggests a differential activation of secretory machinery for translocation of protein during the interaction between *M. mesophilicum* SR1.6/6 and the host plant.

FEMS7-2272
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

GENOME SEQUENCING AND COMPARATIVE GENOME ANALYSIS OF PSEUDOMONAS BALEARICA STRAINS ISOLATED FROM HIGHLY POLLUTED ENVIRONMENTS

F. Salvà-Serra[1], H. E. Jakobsson[1], D. Jaén-Luchoro[1], C. Seguí[2], F. Aliaga[3], A. Busquets[2], M. Gomila[2], J. Lalucat[2], E. R. B. Moore[4], A. Bennasar-Figueras[2]
[1]University of Gothenburg, Department of Infectious Diseases, Gothenburg, Sweden
[2]University of the Balearic Islands, Department of Biology, Palma de Mallorca, Spain
[3]Clínica Rotger, Microbiology, Palma de Mallorca, Spain
[4]University of Gothenburg, Culture Collection University of Gothenburg, Gothenburg, Sweden

Backgrounds

Pseudomonas balearica (former genomovar 6 of *P. stutzeri*) is a marine denitrifying species that has been isolated several times all over the world. In most cases, it was isolated from highly polluted environments, such as oil spills or waste waters. Strains of *P. balearica* have been shown to degrade compounds such as naphthalene, phenanthrene or organophosphorus pesticides.

Objectives

1- To complete the whole-genome sequence of *P. balearica* DSM 6083^T.

2- To obtain the genome sequence of additional strains of *P. balearica*.

3- To compare the genomes of *P. balearica* and strains of related species.

Methods

Genomic DNA of *P. balearica* DSM 6083^T was sequenced with 454 and Illumina technology. The genome was closed combining several bioinformatic methods and Sanger sequencing. Two additional strains of *P. balearica* were sequenced using Illumina and assembled combining *de novo* and reference assemblies. Comparative analysis of four genomes of *P. balearica* and further strains of *P. stutzeri* was done, using UHGene, an *in-house* pipeline that combines different algorithms to determine orthologous gene clusters, that are individually aligned and concatenated to estimate the core- and pan-genome of the species.

Conclusions

The genome of the type strain of *P. balearica* was closed and the genomes of two other strains of the species were obtained as draft. The analysis of the genomes has revealed numerous metabolic pathways and genes that explain the degradative capabilities of the strains and their life-style. Analysis with UHGene has determined the core- and pan-genome of *P. balearica* and supports the current phylogenetic status of the species.

FEMS7-1868
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROORGANISMS AS BIOINDICATORS OF NITROGEN REMOVAL IN ACTIVATED SLUDGE FROM DIFFERENT WASTEWATER TREATMENT PLANTS

E. Ciriero[1], J.A. Parada-Albarracín[2], J. Méndez[3], A.M. Huguet[1], H. Salvadó[2]
[1]Empresa Mixta d'Aigües de la Costa Brava S.A, Operation and maintenance, Roses, Spain
[2]University of Barcelona, Evolutive Biology- Ecology and Environmental Sciences, Barcelona, Spain
[3]University of Barcelona, Fermentation Unit, Barcelona, Spain

Backgrounds

Biological processes in wastewater treatment plants (WWTP) is possible due to a diverse activated sludge biocenosis. Monitoring of certain microorganisms as indicators may offer several advantage in WWTP. Thus, the potential use of microorganisms as bioindicators in the wastewater treatment continues to develop to achieve an understanding of the microbial ecology of activated sludge.

Objectives

Microorganisms potential as indicators of nitrifying performance in different configuration of activated sludge processes were studied.

Methods

Three full-scale WWTP in north west of Catalonia working with different configurations: anaerobic/anoxic/aerobic (A2O), anoxic/aerobic (AO), and oxidation ditch (OD) were used in this study. Sludge samples for microscopic examination in each system were collected from the aeration tanks. Multivariate analysis was performed to correlate the operational and environmental parameters with respect to the microfauna found.

Conclusions

Multivariate analysis showed a cluster involved in ammonia removal efficiency, cluster formed by *Arcella* sp. (0.667), *Calyptotricha lanuginosa* (0.596), *Metacystis* sp. (0.490), *Chaetospira* sp. (0427), *Euglypha* sp. (0.370), and *Pyxidicula* sp. (0.350) showed significant Spearman's correlations coefficients with ammonia removal. Comparative study suggests that high efficiency in nitrogen removal tend to have some protists abundance than others when the efficiency nitrogen removal decreases. Indicating the positive role of *Arcella* sp., *Calyptotricha lanuginosa*, *Metacystis* sp., *Chaetospira* sp., *Euglypha* sp. and *Pyxidicula* sp. have been selected as performance bioindicators based on ammonium removal in this study, highest density of these protozoa was achieved when nitrogen removal was high. We suggest calling this cluster ARI (Ammonium Removal Indicator) as good bioindicators of high nitrogen removal efficiency in WWTP.

FEMS7-0029
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PHARMACEUTICAL AND ENVIRONMENTAL APPLICATION OF A PRODUCT OF BIOLOGICAL ORIGIN AND OTHER SYNTHETICS

F. Samira[1], A. Redha[1], B. Abdelmalek[2]
[1]Yahia FARES University, MEDEA, MEDEA, Algeria
[2]Saad DAHLEB University, BLIDA, BLIDA, Algeria

Backgrounds

The objective of this study was to evaluate firstly, the power of surfactants to remove heavy metals present in the soil, using an extraction method by solvent such as a depollution technique, secondly, to study the stability of pharmaceutical creams using these surfactants. This work has two parts, first, we evaluated the effectiveness of surfactants that are natural (biosurfactant) or synthetic (SDS and Tween 80) on desorption of heavy metals (Ni, Cu).The results were shown that the biosurfactant gave higher recovery yield for Cu, when its concentration is lower than the critical micelle concentration (CMC). In the second part, we studied the stability of an anti-burn cream by these surfactants where it was more stable with Tween 80.

Objectives

1. The study of the effectiveness and the power of surfactant (chemical or biological) in the depollution of the soils rich in heavy metals.
2. The study of the stability of a pharmaceutical cream by various surfactants

Methods

- The analysis technique used throughout this work is: UV-Visible spectrophotometry. It was used to monitor the absorbance of an aqueous solution loaded with heavy metals. The principle of this method is governed by the Beer-Lambert la.
- The pharmaceutical emulsion prepared was subjected to a series of physico-chemical analysis, namely macroscopic appearance, pH, microscopic appearance, emulsion direction and stability. These analyzes are necessary to evaluate the effect of each surfactant on the stability of the surfactant

Conclusions

1. As a result, it was concluded that the best depollutant was the biosurfactant because the natural surfactants are biodegradable and non-toxic to synthetic surfactants
2. The surfactants having a liquid form are easier to use and play an emulsifying role in the dispersed systems and it is subsequently concluded that Tween 80 promotes a uniform distribution of The cream

FEMS7-0714
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ROLE OF CHEMOTAXIS IN THE COLONIZATION OF SALICORNIA PLANTS WITH HALOPHILIC BACTERIA

I. Sampedro[1,2], *L. Toral*[1,2,3], *I. Llamas*[1,2]
[1]*Pharmacy Faculty, Microbiology, GRANADA, Spain*
[2]*Biomedical Research Center CIBM- Biotechnology Institute, Microbiology, GRANADA, Spain*
[3]*Xtrem Biotech S.L.- European Business Innovation Center, Microbiology, GRANADA, Spain*

Backgrounds

Based on FAO statistics, 800 million hectares of land are degraded through drought in the world. Climate change will affect the sea level increasing the area of wetland ecosystems and threaten the agriculture production by the increase of soil salinity.Therefore, the halophiles plants growth promotion in saline soils is an important strategy to consider. The halophilic plant *Salicornia* is one of the most promising biotic resources for the economic development of semiarid regions. Regarding to the chemotaxis, it has been extensively described in bacteria such as *Escherichia coli*, however, it is essentially unknown for halophilic bacteria.

Objectives

The main objective of this study is the characterization of the bacteria associated with *Salicornia* and their chemotaxis.

Methods

Bacteria isolated from *Salicornia* plants were identified by sequencing their 16S rRNA gene. Chemotaxis was quantified using the Adler capillary assay.

Conclusions

The bacteria associated with *Salicornia* belong mainly to the *Halomonas* genus. We have found that *Salicornia* exudates are chemoattractants for *H. anticariensis*. HPLC analysis shows the presence in *Salicornia* exudates of macrolides like Calenduloside E and it derivate oleanolic acid, a triterpenoid widely distributed in plants. We also showed that oleanolic acid is a chemoattractant for the halophilic bacteria used in this study. Inoculation of *Salicornia* seeds with *H. anticariensis* resulted in significant increases of germination percentage and vigour index compared with the results obtained in seeds inoculated with the chemotaxis mutant. These studies suggest the positive influence of chemotaxis in the colonization of *Salicornia* plants with halophilic bacteria.

FEMS7-0868
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ANTIFUNGAL ACTIVITY OF LIPOPEPTIDES FROM BACILLUS SP. AGAINST BOTRYTIS CINEREA

L. Toral[1,2,3], V. Béjar[1,2], I. Sampedro[1,2]
[1]Pharmacy Faculty, Microbiology, GRANADA, Spain
[2]Biomedical Research Center CIBM- Biotechnology Institute, Microbiology, GRANADA, Spain
[3]Xtrem Biotech S.L.- European Business Innovation Center, Microbiology, GRANADA, Spain

Backgrounds

Botrytis cinerea is a filamentous fungus classified as the second most important phytopathogen worldwide. Due to the low efficiency of control strategies against this fungus it causes important economic and agricultural losses in vineyards, and in tomato and strawberry crops.

Objectives

The main objectives of this study are to characterize the antifungal activity of strain Bacillus sp., against B. cinerea and to identify the compounds that are responsible for its activity.

Methods

In vitro techniques antibiosis, microscopy studies, determination of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC), identification and characterization of lipopeptides using PCR and electrospray Q-TOF mass spectrometry.

Conclusions

The Bacillus sp. strain exhibited antifungal activity against B. cinerea in solid and liquid medium (inhibition rates of 60% and 100%, respectively). Microscopy studies revealed a morphological alteration of phytopathogen in interaction with Bacillus sp. strain. Molecular and mass spectrometry analyzes confirmed that Bacillus sp. secretes various forms of surfactin, iturin/mycosubtilin and fengycin. We demonstrate that these are the main compounds that have a bio-control ability.

FEMS7-1552
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INSIDE OUT: ARE GUT BACTERIA INVOLVED IN WHITEFLIES' PLANT ADAPTATION?

D. Santos-Garcia[1], N. Mestre-Rincon[1], E. Zchori-Fein[2], S. Morin[1]
[1]The Hebrew University of Jerusalem - The Faculty of Agriculture, Entomology, Rehovot, Israel
[2]Newe Ya'ar Research Center- The Agricultural Research Organization, Entomology, Ramat Yishay, Israel

Backgrounds

Polyphagous insects require an ability to overcome different sets of host plant defense compounds. Usually, during host-plant switch, their performance decline and only rebound few generations later. In contrast to natural selection, phenotypic plasticity and new gut bacteria acquisition are fast processes that could be involved in the host switching process.
The whitefly *Bemisia tabaci*, a tiny phloem-feeder, is considered an important and polyphagous pest species. Reported microbiomes of *B. tabaci* are dominated by obligatory bacterial endosymbionts. However, because insects' gut microbiota can play a major role in host-plant adaptation and are mainly acquired from the diet/environment, we hypothesized that *B. tabaci* might acquire environmental bacteria that can provide different benefits/costs related to host adaptation.

Objectives

Our goal was to explore the putative role of gut bacteria in the plant-adaptation process of *B. tabaci* under field conditions.

Methods

Field-like assays were conducted by following the adaptation process of a *B. tabaci* population switching from watermelon (suitable host) to pepper (unsuitable) along four generations. Each generation, samples were collected for gut dissections (cDNA 16S rRNA amplicons) and performance assays (offspring survival). Amplicons were amplified using endosymbionts' dual priming blockers and analyzed using USEARCH and phyloseq.

Conclusions

B. tabaci adaptation to pepper took 2-3 generations. Microbiome differences between *B. tabaci* watermelon/pepper populations were significant. Genera significantly associated with watermelon or pepper diets were identified. Also, a generational effect was detected, being more noticeable in the pepper samples. These data suggest that gut bacteria might help *B. tabaci* to adapt to new host plants.

FEMS7-1561
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

USING PORTIERA TO ANSWER OPEN QUESTIONS IN WHITEFLIES TAXONOMY

D. Santos-Garcia[1], N. Mestre-Rincon[1], N. Netanel[2], D. Ouvrard[3], E. Zchori-Fein[2], S. Morin[1]
[1]The Hebrew University of Jerusalem - The Faculty of Agriculture, Entomology, Rehovot, Israel
[2]Newe Ya'ar Research Center- The Agricultural Research Organization, Entomology, Ramat Yishay, Israel
[3]Natural History Museum, Department of Life Sciences, London, United Kingdom

Backgrounds

Whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae) are small phloem feeding insects that include several agricultural pests. In contrast to other insects, whiteflies taxonomy is based on nymphal stages morphology. However, this stage shows phenotypic plasticity, which produces several inconsistencies at the genus level.
Whiteflies harbor specialized cells that host an obligatory/primary bacteria endosymbiont named *Candidatus* Portiera aleyrodidarum which complements the insect's diet. *Portiera* has been vertically transmitted since the divergence of whiteflies from their Psyllinea ancestor (*circa* 160 Mya), and therefore can reflect its host phylogeny. Also, *Portiera* from *Bemisia tabaci* lineage has lost the polymerase proofreading sub-unit (*dnaQ*) and presents an uncommon genome instability.

Objectives

Our main goal was to establish a phylogenetic framework based on several *Portiera*'s genes. This framework will be used to improve whiteflies classification and to trace the *dnaQ* loss and the rise of genomic instability.

Methods

A PCR screening with specific primers targeting five *Portiera* genes (*16S* and *23S* rRNA, *groEL*, *dnaK, rpoD* and *dnaQ*), plus some *Portiera* rearrangements, was performed on 22 whiteflies species (including *exsiccata* museum specimens). Phylogenetic methods were used for tree inference, divergence dating, species delimitation and ancestral node reconstruction of *dnaQ/*rearrangements (RaxML, BEAST2, bPTP and ape).

Conclusions

The use of *Portiera* sequences outperform the universal mitochondrial cytochrome oxidase subunit I (mtCOI) region as they show low signal saturation, avoid common misclassification of similar insects and can be used on parasitized samples. Therefore, it solves some problems/inconsistencies in current whiteflies phylogeny. The loss of *dnaQ* and the associated rearrangements are unique to the *Bemisia* genus.

FEMS7-2528
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIOME OF THE MAIZE RHIZOSPHERE CULTIVATED WITH ROCK PHOSPHATE BY CULTURE-DEPENDENT AND INDEPENDENT APPROACHES

V. Santos[1], U. Silva[1], D.R.C.D. Silva[1], L.F. Freitas Jr.[1], A.C. Fernandes[1], J.D. Medeiros[2], S.C. Orellana[2], C.A. Oliveira[3]
[1]Biological Sciences Institute, Microbiology, Belo Horizonte, Brazil
[2]Research Center René Rachou/FIOCRUZ, Group of Genomics and Computational Biology, Belo Horizonte, Brazil
[3]Embrapa Milho e Sorgo, Microbiology, Sete Lagoas, Brazil

Backgrounds

Maize (Zea mays L.) is a crop used worldwide. For its productivity is necessary use of high quantity of fertilizers. For greater agricultural sustainability, has been investigated use of rock phosphate (RP) of low reactivity together with microbial inoculants capable phosphorus (P) providing of these rocks. However, little is known about community of RP solubilizing microorganisms, specially, by culture-independent techniques.

Objectives

To access the microbial diversity on the maize rhizosphere cultivated in soil fertilized with RP, Triple superphosphate (TSP) or no P using new generation sequencing.

Methods

Amplicons of ribosomal markers (16S rRNA V3-V4 and ITS regions) were sequenced on Miseq Illumina platform and mapped against the Greengenes and Unite databases. For isolation of the PSB, it was used enrichment cultures using NBRIP broth supplemented with RP and added of rizhospheric soil from area fertilized with RP or no P fertilized as inoculum, followed selection on the NBRIP agar added calcium triphosphate.

Conclusions

Taxa related to P solubilization (PSB) were enriched in the soil RP added, including *Burkholderia* sp. and *Bacillus* sp., while *Klebsiella s*p. was enriched in the soil without P addition. For fungal community, mycorrhizal fungi predominated in the soil RP added, such as *Scutellospora* sp., *Racocetra* sp., *Acaulospora* sp. 32 strains of PSB were obtained in the enrichment culture, with predominance of *Klebsiella* in the RP soil. The strains 20 and 21 of this genus showed high RP and iron phosphate solubilization potential correlating with lower pH values. Some bacteria also showed capacity to produce siderophore and to antagonize phytopathogens.

FEMS7-2565
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

TAXONOMIC AND FUNCTIONAL DIVERSITY OF THE BACTERIAL COMMUNITY ASSOCIATED TO THE LIQUID FRACTIONS OF AQUIFER CONTAMINATED WITH CREOSOTE THROUGH METATAXONOMIC ANALYZES.

V. Santos[1], A.D.L. Júlio[1], F.S. Castanon[1], U.D.C. Silva[1], J.M. Dutra[2], D.K. Morais[2], S.C. Orellana[2]
[1]Biological Sciences Institute, Microbiology, Belo Horizonte, Brazil
[2]Research Center René Rachou/FIOCRUZ-, Group of Genomics and Computational Biology, Belo Horizonte, Brazil

Backgrounds

Bioremediation has been considered a cost-effective and environmentally friendly method to remediate contaminated areas, as by creosote, which consists predominantly of Polycyclic Aromatic Hydrocarbons. However the efficiency of technique is dependent of knowledge of taxonomic and functional diversity of the microbiota on these sites.

Objectives

In this study, we used metataxonomic analyzes to characterize the bacterial community from samples of aquifer liquid fraction contaminated with creosote and to predict its catabolic potential.

Methods

Samples were collected from eight points with different levels of contamination in Espírito Santo State-Brazil. The DNA of the samples was extracted using the PowerWatter DNA Isolation Kit, and the 16S rRNA amplicons of bacterial ribosomal markers (16S rRNA V3-V4 regions) were sequenced on the IlluminaMiseq platform.

Conclusions

A negative correlation was observed between the alpha diversity of the samples and the concentration of the contaminants. At the phylum level, it was observed a predominance of Proteobacteria in all the samples, but others taxonomical levels varied widely. The most enriched taxa in the contaminated samples were the genera *Treponema, Geobacter, Bdellovibrio, Hydrocarboniphaga, Anaerolinea, Paulidibacter, Comamonas* and *Desulfomonille.* Regarding beta diversity, a group consisting of non-contaminated samples and a group formed by the majority of the highly-contaminated samples was formed, and a greater proportion of xenobiotic degradation pathways were observed in the contaminated samples. The data indicate the presence of microorganisms with catabolic potential in the area, which can be biostimulated *in situ* to accelerate the degradation of contaminants.

FEMS7-0979
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DIVERSITY OF CULTURED BACTERIA FROM THE DEEP OCEAN

I. Sanz Sáez[1], *S. G. Acinas*[1], *O. Sánchez Martínez*[2]
[1]*Institut de Ciències del Mar - CSIC, Biologia Marina i Oceanografia, BARCELONA, Spain*
[2]*Universitat Autònoma de Barcelona. Facultat de Biociències,*
Departament de Genètica i Microbiologia, Barcelona, Spain

Backgrounds

Isolation of marine microorganisms is fundamental to infer their physiology, ecology and genome content. However, little effort has been invested to isolate microorganisms from the deep sea, and previous works have focused on the use of molecular methodologies or have been carried out at a local scale.

Objectives

To fill the existing gap concerning the research of microorganisms from the deep ocean using culture dependent techniques two main objectives were addressed: (i) to describe the identity and diversity of 1317 isolates retrieved from bathypelagic sea samples, and (ii) to compare the phylogenetic relationship of these deep ocean isolates with cultures obtained from photic waters of the same oceanographic locations.

Methods

We isolated 1259 bathypelagic isolates from the Mediterranean Sea (2000 m depth), and North and South Atlantic Ocean (4000 m depth) using different culture media to maximize the diversity retrieval. Additionally, a total of 1097 isolates were also isolated from photic waters from the same samples (NW Mediterranean, North and South Atlantic Ocean), as well as from Indian and Arctic Oceans for comparative analyses. The 16S rRNA gene was partially sequenced for a total of 1317 isolates (surface and deep) to infer their phylogenetic relationship.

Conclusions

The isolates retrieved from the deep ocean were mainly affiliated to the phylum Proteobacteria, (classes Alphaproteobacteria and Gammaproteobacteria), and Bacteroidetes. A large proportion (54.6%) of the deep isolates sequences were 100% identical to sequences of previous identified photic isolates, highlighting the cosmopolitan nature of these bacteria in the whole water column. In addition, two isolates, related to the genera *Teredinibacter* sp. and *Mesonia* sp. may represent new potential genera.

FEMS7-0442
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL DIVERSITY IN HEAVY METAL POLLUTED SOILS ASSESED VIA CULTURE-DEPENDENT APPROACH

G. Satchanska[1], A. Doicheva[1], V. Groudeva[2], E. Golovinsky[3]
[1]New Bulgarian University, Natural Sciences, Sofia, Bulgaria
[2]Sofia University, Faculty of Biology, Sofia, Bulgaria
[3]Institute of Molecular Biology-Bulgarian Academy of Sciences, Biochemical pharmacology and molecular design, Sofia, Bulgaria

Backgrounds

Heavy metal polluted soils represent one of the most severe environmental problems. Metal ions as Hg^{2+}, Cd^{2+}, Ag^{+}, Cu+, Zn^{2+} and Pb^{2+} form strong toxic complexes in the biological cells, bind to enzymes and erythrocytes, disrupt the cell membrane and are responsible for genotoxic effects. Bacteria are highly sensitive and adaptive to pollutants organisms and could serve as reliable biomarker for environmental contamination.

Objectives

Shifting of the microbial community structure was detected in three metal-polluted soils collected near the Pb-Zn smelter KCM and pesticide enterprise AGRIA, Bulgaria. Due to new waste water treatment plant functioning the environmental pollution was significantly decreased.

Methods

CFU of heterotrophic aerobs, heterophic anaerobs, sporeforming bacteria, denitrifying bacteria, amonifying bacteria, nitrifying bacteria, Fe(II)-oxidizing bacteria, Mn(II)-oxidizing bacteria, Fe(III)-redicing bacteria, Mn(IV)-reducing bacteria, colourless sulphur bacteria, cellulose degradating bacteria, oligocarbophiles, actinomicetes and fungi were assessed *via* culturing on selective media. Presence of metal-leaching bacteria as *A. thiooxidans*, *A. ferrooxidans*, *A. denitrificans*, A. *thioparus* was analyzed as well. Twenty novel indigenous soil bacterial isolates were cultured. Most of them showed metal resistance.

Conclusions

Microbial community structure in two of investigated soil samples was affected due to heavy metal and pesticide pollution. Our results demonstrated the nitrifying and denitrifying bacteria as well as actinomycetes and fungi were not detected in two out of three samples. Absence of above mentioned bacterial groups resulted in abundance of other groups as heterotrophic and sporeforming bacteria. Microbial community responded to long-term heavy metal- and pesticide contamination through changes in its structure and selection of metal resistant bacteria.

FEMS7-3217
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SACA LA LENGUA: A STUDY OF THE HUMAN MOUTH MICROBIOME

L. Cozzuto[1,2], J. Willis[1,2], M. Alloza[1,2], N. Andreu Somavilla[1,2], C. Company[1,2], A. Hermoso Pulido[1,2], A. Pittis[1,2], E. Ksiezopolska[1,2], S. Iraola[1,2], E. Saus[1,2], H. Onywera[1,3], C. Carolis[1,2], J. Hecht[1,2], J. Ponomarenko[1,2], T. Gabaldón[1,2]
[1]Centre for Genomic Regulation CRG, Bioinformatics and Genomics, Barcelona, Spain
[2]Universitat Pompeu Fabra, UPF, Barcelona, Spain
[3]Institute of Infectious Disease and Molecular Medicine IDM, University of Cape Town UCT, Cape Town, South Africa

Backgrounds

High-throughput DNA sequencing continues to offer comprehensive insights into microbial ecosystems and we utilize this technology here to explore human oral microbiome of about 1500 teenagers throughout Spain in the citizen's science project "Saca La Lengua". Several bioinformatics tools have been inconclusively benchmarked, yet variations in algorithms are known to impact the microbiome results.

Objectives

First, we aimed to validate 16S rRNA sequencing and four bioinformatics tools for microbiome analyses. Then, we applied this technology to explore the oral microbiome of Spanish students around age 15 and analyze it alongside survey data regarding daily habits, hygiene, diet, location and others.

Methods

Genomic DNA from two microbial mock communities was sequenced by shotgun and V3-V4 16S rRNA sequencing. Four bioinformatics tools for 16S rRNA analysis – mothur, QIIME, QUPARSE and riboPicker – were set up and tested. 1582 "Saca La Lengua" samples were collected, and data obtained by 16S sequencing was processed with the mothur pipeline. Collected survey data provided by each individual and metadata associated with each sample was used to perform correlative analyses with the sample counts.

Conclusions

Species abundances were significantly different between 16S and WGS approaches, although genera distributions by most tools were similar to the 16S mapping data. Overall, mothur performed better than the other three bioinformatics tools tested. In "Saca La Lengua" samples, *Streptococcus* was generally the most abundant genus, while several correlations were found between samples and survey, as well as within samples, indicating numerous factors influencing the growth of many genera.

FEMS7-0843
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

METAGENOMIC ANALYSIS OF HOT SPRINGS IN CENTRAL INDIA REVEALS HYDROCARBON DEGRADING THERMOPHILES

R. Saxena[1], D.B. Dhakan[1], P. Mittal[1], P. Waiker[1], A. Chowdhury[2], A. Ghatak[2], V.K. Sharma[1]
[1]Indian Institute of Science Education and Research, Department of Biological Sciences, Bhopal, India
[2]Indian Institute of Science Education and Research,
Department of Earth and Environmental Sciences, Bhopal, India

Backgrounds

Extreme ecosystems such as hot springs are of great interest as a source of novel extremophilic species, enzymes and biotechnological products. India harbors hundreds of hot springs, the majority of which are not yet explored and require comprehensive studies to unravel their unknown phylogenetic and functional diversity.

Objectives

The aim of this study was to perform a large-scale metagenomic analysis of three major hot springs located in central India namely, Badi Anhoni, Chhoti Anhoni and Tattapani, to uncover their resident microbial community and functional traits.

Methods

Water samples were collected from seven distinct sites of the three hot spring locations with temperature ranging from 43.5-98°C. 16S rRNA gene amplicon sequencing of V3 hypervariable region and shotgun metagenome sequencing was carried out.

Conclusions

Taxonomic and functional analysis revealed hydrocarbon degradation and methane metabolism pathways to be abundant in the Anhoni hot springs (43.5-55 °C), dominated by *Methylococcus capsulatus*, *Pseudomonas stutzeri* and *Acidovorax* sp., suggesting the presence of a chemoorganotrophic thermophilic community at Anhoni. The Tattapani hot spring with a high-temperature range (61.5-98 °C) displayed a lower microbial diversity, and was primarily dominated by a nitrate-reducing archaeal species *Pyrobaculum aerophilum*. A higher abundance of cell metabolism pathways essential for the microbial survival in extreme conditions was observed at Tattapani. A novel consortium of microbes, genes and pathways associated with the prominent hot springs of India having potential applications in biotechnology and bioremediation were revealed. The main results of this study will be presented.

FEMS7-2390
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IDENTIFICATION OF THOUSANDS OF CONSERVED SMALL PROTEINS WIDESPREAD IN MICROBIAL METAGENOMES

H. Sberro Livnat[1], *A. Bhatt*[1]
[1]*Stanford University, Genetics and Medicine, Palo Alto, USA*

Backgrounds

Small bacterial proteins (<=50 aa) have traditionally been overlooked due to the difficulty of distinguishing proteins encoding open reading frames (ORFs) from those occurring by chance. However, the few examples that have been characterized, indicate that small proteins play important roles in bacterial physiology, including morphogenesis, cell division, transport, enzymatic activities and more.

Objectives

Here, we took advantage of the growing wealth of metagenomic data to systematically detect small ORF (sORFs) within hundreds of metagenomic samples originating from diverse metazoan hosts as well as environmental samples.

Methods

For each metagenome, sORFs were recovered using *ab initio* gene finding algorithms with adjusted parameters. Clustering of these sORFs based on sequence similarity revealed families that are conserved and widespread in microbial metagenomes.

Conclusions

Our analyses, while retrieving some of the well-characterized small proteins including lineage specific proteins such as SpoVM, SgrT, AcrZ, CydX, PmrR, Blr, MntS, KdpF and others that are found in diverse lineages, such as small ribosomal proteins, have also exposed **thousands of yet unknown conserved sORFs**. Within this set, we could identify ~100 putative secreted protein families and ~2000 putative transmembrane families, possibly representing novel mediators between bacteria and their environment. Furthermore, we discriminate between sORF families that are enriched in host-associated metagenomes and those that are also found in environmental microbes. Whereas proteins in the first group may be important for adaptation of bacteria to life within a metazoan host, short proteins that are found in diverse ecological niches are more likely to display ‘house-keeping’ roles.

FEMS7-0838
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECT OF MULTIPLICITY OF INFECTION, TEMPERATURE AND PH ON THE ACTIVITY OF ISOLATED PSEUDOMONAS AERUGINOSA BACTERIOPHAGES

G. Scarascia[1], S. Yap[1], P. Hong[1]
[1]King Abdullah University of Science & Technology KAUST,
Biological and Environmental Science and Engineering, Thuwal Jeddah, Saudi Arabia

Backgrounds

Pseudomonas aeruginosa is a thiosulfate-reducing bacteria ubiquitous in the marine environment. Their presence is often associated with membrane biofouling and biocorrosion in desalination process. Chlorine is commonly used as biocide against *P. aeruginosa* but would require sodium metabisulfite to neutralize residual content. High sulfate content in seawater, coupled with the addition of metabisulfite can further select for *P. aeruginosa* and/or other sulfate-reducers. Bacteriophage treatment can be used to complement current biocidal strategies.

Objectives

This study aims to assess the optimal conditions (i.e., multiplicity of infection (MOI), temperature, pH) for phage treatment against *P. aeruginosa*.

Methods

Optical density of the *P. aeruginosa* cultures was monitored over 24 h in presence of isolated bacteriophages. UF membranes were retrofitted into drip flow reactors, and seawater biofilm was allowed to develop on the membranes prior to phage application.

Conclusions

In co-culture with phages, *P. aeruginosa* growth was characterized by an initial lag phase followed by growth. Non-infected cultures were characterized by a lag-phase of between 0 and 2h; the lag-phase in presence of phage at MOI of 10 and 1 was 10h and 7h, respectively. A MOI of 0.1 achieved lag phase of 3h but the growth rate was significantly lower than that observed at MOI 10 and 1 (p<0.0001).
The growth rates of non-infected cultures were very similar over the range of temperature (25-45°C). However, the presence of phage resulted in a reduced growth rate at 25°C compared to 30°C and 37 °C (p<0.0001), suggesting that *P. aeruginosa* is more sensitive to low temperature upon bacteriophages exposure. Similarly, phage treatment of biofilm at 25°C showed stronger reduction of CFU compared to the control and to the other temperatures (p<0.0001).
All bacteriophages exhibited similar lytic effect over pH 5.0-8.0, revealing that their application can be coupled with conventional biocides or cleaning strategies.

FEMS7-0841
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DETECTING TOXIC CYANOBACTERIA IN BRAZILIAN FRESHWATER RESERVOIRS USING NEW PIGMENT/CHLOROPHYLL A RATIOS

L. Schlüter[1], G.S. David[2], N.O.G. Jørgensen[3], R. Podduturi[3]
[1]DHI, Environment & Toxicology, Hørsholm, Denmark
[2]São Paulo State Agribusiness Agency, APTA, Jau, Brazil
[3]University of Copenhagen,
Section of Microbial Ecology and Biotechnology- Department of Plant and Environmental Sciences, Copenhagen, Denmark

Backgrounds

Intensified use of water bodies and reservoirs for e.g., aquaculture increases the need for monitoring and early warning of toxins from cyanobacteria, and simple analytical monitoring methods, which can determine this risk, are crucial.

Objectives

The content of pigments and the toxic microcystins of different strains of cyanobacteria at three different light intensities and at stationary growth was investigated to determine if pigment analyses can be used for determining presence of toxic cyanobacteria in reservoirs.

Methods

The pigments and microcystins were analysed by HPLC. The pigment/chlorophyll (Chl) *a* ratios were tested using the CHEMTAX software to calculate the biomass of phytoplankton groups in samples from four sampling periods in five Brazilian reservoirs.

Conclusions

Various different carotenoids characteristic for cyanobacteria were detected in the cultures: two different myxoxanthophylls (myxol glucosides), echinenone, canthaxanthin, β-cryptoxanthin, oscillaxanthin, aphanizophyll, nostoxanthin, caloxanthin, as well as zeaxanthin and β-carotene. The diagnostic pigments, echinenone and myxoxanthophyll, were commonly present in the cultured cyanobacteria and in the reservoirs. The presence of two types of cyanobacteria in the reservoirs were determined from the pigment concentrations and ratios between the pigments: filamentous/colony forming cyanobacteria with echinenone and myxoxanthophyll, and coccoid/pico-sized cyanobacteria with zeaxanthin only. New pigment/Chl *a* ratios were used to calculate the biomass of toxic cyanobacteria as well as other phytoplankton groups. The concentrations of microcystins were significantly correlated to the Chl *a* concentrations of cyanobacteria, and indicated that pigment analysis by HPLC can be used as early warning for detecting microcystin-producing cyanobacteria in the Brazilian reservoirs investigated.

FEMS7-3187
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE CRISPR-CAS GENE CLUSTER IN BURKHOLDERIA GLUMAE STRAIN PG1 IS IN PART CONTROLLED BY ITS THREE N-ACYL-HOMOSERINE-LACTONE QUORUM-SENSING SIGNALS

C. Schmeisser[1], *G. Stiebelehner*[1], *W.R. Streit*[1]
[1]*Biocenter Klein Flottbek, Microbiology and Biotechnology, Hamburg, Germany*

Backgrounds

Burkholderia glumae PG1 (BG1) is a soil-associated motile plant pathogenic bacterium. Recently we have established the genome sequence of this organism. BG1 encodes a CRISPR-cas gene cluster of the F-I (Yersinia pestis) subytpe. Using RNA-seq we observed that the expression of the cas/csy genes is regulated in a quorum sensing-dependent manner. Thereby it is notably that BG1 codes for three autoinducer (AI) synthases (bgal1-bgal3), which are all involved in the synthesis of N-acyl-homoserine-lactones (AHLs) with varying chain length. This is an unusual feature within the species *B. glumae*, since the genomes of all other currently sequenced strains contain only a single AHL synthase gene. Mutations in each of the three loci resulted in the up to 10-fold reduced expressions of the respective cas/csy genes on a population wide level.

Objectives

Our aim is to identify the role of CRISPR-cas in BG1 besides its obvious role for immunity. Further we are interested in identifying the key regulators involved in this QS-dependent response and the level of AI dependence. Finally, we are searching for a possible link between the CRISPR-cas gene expression and the interaction with the host plant.

Methods

To reach our aims we used RNA seq, qRT-PCR and promoter fusion approaches.

Conclusions

The work demonstrated that quorum sensing in BG1 affects CRISPR-cas gene regulation. It also provides evidence that the cas gene expression depends on the presence of oxo-C_8 AHL and is less effected by oxo-C_{10} and oxo-C_{12} AHL. This indicates a complex mode of regulation.

FEMS7-1508
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

AN INTEGRATED OMICS ANALYSIS OF THE RUMEN MICROBIAL COMMUNITY IN SHEEP FED BACILLUS AMYLOLIQUEFACIENS H57

B. Schofield[1], Y.K. Yeoh[2], O.T. Le[3], N. Lachner[2], D. McNeill[3], D. Ouwerkerk[4], P. Dart[1], A. Klieve[5], P. Hugenholtz[2]
[1]The University of Queensland, School of Agriculture and Foods Sciences, St Lucia, Australia
[2]The University of Queensland,
Australian Centre for Ecogenomics- School of Chemistry and Molecular Biosciences, St Lucia, Australia
[3]The University of Queensland, School of Veterinary Science, Gatton, Australia
[4]Queensland Government, Agriscience Queensland- Department of Agriculture and Fisheries, Dutton Park, Australia
[5]The University of Queensland, School of Agriculture and Foods Sciences, Gatton, Australia

Backgrounds

The probiotic *Bacillus amyloliquefaciens* strain H57 increased weight gain, feed intake and nitrogen retention when fed to pregnant Dorper ewes.

Objectives

We undertook a metagenomic and metatranscriptomic analysis of the rumen microbiome to elucidate possible mechanisms for the probiotic response.

Methods

The relative dominance of particular population genomes, assembled from the rumen metagenome, differed between the Control sheep and those fed H57. For the Control sheep, a *Prevotella sp.* (SR_c51) and a *Succinivibrionaceae sp.* (SR_c46) were dominant, while in the H57 fed sheep it was two *Prevotella* spp. (SR_t35 and SR_t60) that dominated. A gene-centric analysis of the transcripts that mapped to population genomes in both Control and H57 fed sheep showed the population genomes clustered into functional guilds that were based on taxonomy. A number of gene families involved with the degradation of hemicellulose were significantly more abundant in the metatranscriptomes of H57 fed sheep. Mapping the reads associated with these gene families to the population genomes showed that *Lachnobacterium sp.* (SR_t49), *Prevotella sp.* (SR_t35) and *Prevotella sp.* (SR_t60) were the populations most responsible for expression of genes associated with hemicellulose degradation in the H57-fed rumen microbiome.

Conclusions

B. amyloliquefaciens H57 fed to sheep elicited a shift in the dominant rumen microbiome towards populations expressing a greater abundance of genes associated with hemicellulose degradation. Such an increased ability to digest plant cell wall components may support the increased weight gain observed in the H57 fed sheep. Supported by ARC Linkage Grant with Ridley AgriProducts.

FEMS7-3260
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ROLE OF TYPE II SECRETION/TYPE IV PILUS ASSEMBLY COMPLEXES IN SELF-SUPPRESSION OF CYANOBACTERIAL BIOFILM DEVELOPMENT

R. Schwarz[1], E. Nagar[1], R. Parnasa[1], Y. Yegorov[1], Z. Reich[2], S. Zaidman[3], R. Simkovsky[4], S. Golden[4], E. Sendersky[1]
[1]Bar-Ilan University, Faculty of Life Sciences, Ramat Gan, Israel
[2]Weizmann institute of Science, Department of Biological Chemistry, Rehovot, Israel
[3]Weizmann institute of Science, Electron Microscopy Unit, Rehovot, Israel
[4]University of California, Division of Biological Sciences, San Diego, USA

Backgrounds

Information on the molecular mechanisms underlying biofilm formation in cyanobacteria is scarce in spite of their environmental prevalence and the economic loss associated with these microbial assemblages. We revealed a self-suppression mechanism that prevents biofilm development in the cyanobacterium *Synechococcus elongatus*.

Objectives

Characterization of the role of type II/type IV secretion systems in the self-suppression mechanism of cyanobacterial biofilm development.

Methods

Genetic, mutational, electron microscopy and physiological approaches.

Conclusions

We demonstrated that the planktonic nature of the cyanobacterium *Synechococcus elongatus* is a result of a self-inhibition mechanism, which depends on the deposition of factor(s) to the extracellular milieu. These extracellular inhibitory substances govern expression of small secreted proteins that enable biofilm development. Inactivation of a gene encoding a homolog of the ATPase subunit of type II protein secretion or type IV pilus assembly systems impairs the inhibitory process and results in biofilm formation (1-3). Additionally, the RNA-chaperone homolog, Hfq, and a highly conserved cyanobacterial protein, thus far annotated as hypothetical, are essential for the biofilm inhibitory mechanism. Furthermore, inactivation of either one of the genes encoding these proteins, results in aberrant protein secretion and absence of pili, suggesting involvement of the Hfq-homolog and the "hypothetical protein" in modulation of type II/type IV complexes. Localization of these modulators to the photosynthetic membranes implies a new mode of regulation of type II/Type IV systems in cyanobacterial cells.

1. Schatz et al. (2013) Environ Microbiol, 15(6), 1786-1794.
2. Nagar and Schwarz (2014) Environ Microbiol. 17(5):1477-1486.
3. Parnasa et al. (2016) Sci. Rep. 6:32209

FEMS7-2361
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL COMMUNITIES OF HEAVY OIL RESERVOIRS

E. Semenova[1], D. Sokolova[1], T. Babich[1], I. Borzenkov[1], A. Ershov[1], T. Tourova[1], A. Poltaraus[2], T. Nazina[1]
[1]Winogradsky Institute of Microbiology- Research Center of Biotechnology RAS, Laboratory of Petroleum Microbiology, Moscow, Russia
[2]Engelhardt Institute of Molecular Biology- RAS, Laboratory of structural and functional genomics, Moscow, Russia

Backgrounds

In many oil-producing countries, deposits of light conditional oil have already exhausted, so it is important to develop technology of heavy oil (with a density greater than 0.92 g/cm^3) recovery. Geological reserves of heavy and high-viscosity oil in Russia reached 6–7 billion tons, but their extraction requires expensive technologies. Biological effect on heavy oil is selective for specific reactions, improving its characteristics, and may represent an alternative approach for additional oil recovery. Currently, information on the microorganisms inhabiting of heavy oil reservoirs remains fragmented.

Objectives

The goal of the work is determination of functional and phylogenetic diversity of microorganisms in heavy oil reservoirs and their biotechnological potential for enhancement of heavy oil recovery.

Methods

Microbial community composition of formation water samples from heavy oil reservoirs (Russian Federation) was studied by radioisotope and culture-based techniques, by high-throughput sequencing of the 16S rRNA genes and DGGE analyses of *mcrA* genes of methanogens.

Conclusions

It was revealed that in oilfields exploited without water-flooding microbial populations were scarce, while microbial numbers in water-flooded oilfields were as high as 10^6 cells/mL. The rates of sulfate reduction and methanogenesis were low. The library of 16S rRNA genes included sequences of bacteria *Acinetobacter*, *Pseudomonas, Dechloromonas* and *Rhodococcus* genera and of methanogenic archaea. Members of genera *Acinetobacter*, *Pseudomonas*, *Bacillus* and *Gordonia*, capable of biosurfactant production, were isolated from formation waters. Aerobic organotrophic and anaerobic fermentative bacteria, revealed in oil reservoir, produce biomass and metabolites (volatile acids and alcohols) having the oil-displacing properties.

This work was supported by Russian Science Foundation, grant no. 16-14-00028.

FEMS7-3213
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SMALL SECRETED PROTEINS ENABLE BIOFILM DEVELOPMENT IN THE CYANOBACTERIUM SYNECHOCOCCUS ELONGATUS

E. Sendersky[1], R. Parnasa[1], E. Nagar[1], Z. Reich[2], R. Simkovsky[3], S. Golden[3], R. Schwarz[1]
[1]Bar-Ilan University, Faculty of Life Sciences, Ramat Gan, Israel
[2]Weizmann Institute of Science, Department of biomolecular sciences, Rehovot, Israel
[3]University of California, Division of Biological Sciences, San Diego, USA

Backgrounds

Small proteins characterized by a double-glycine (GG) secretion motif, typical of secreted bacterial antibiotics, are encoded by the genomes of diverse cyanobacteria, but their functions have not been investigated to date.

Objectives

Using a biofilm-forming mutant of *Synechococcus elongatus* PCC 7942 and a mutational approach, we demonstrate the involvement of four small secreted proteins and their GG-secretion motifs in biofilm development. These proteins are denoted EbfG1-4 (enable biofilm formation with a GG-motif). Furthermore, the conserved cysteine of the peptidase domain of the Synpcc7942_1133 gene product (dubbed PteB for peptidase transporter essential for biofilm) is crucial for biofilm development and is required for efficient secretion of the GG-motif containing proteins. Transcriptional profiling of *ebfG*1-4 indicated elevated transcript levels in the biofilm-forming mutant compared to wild type (WT). However, these transcripts decreased, acutely but transiently, when the mutant was cultured in extracellular fluids from a WT culture, and biofilm formation was inhibited.

Methods

mutational approach

Conclusions

We propose that WT cells secrete inhibitor(s) that suppress transcription of *ebfG*1-4, whereas secretion of the inhibitor(s) is impaired in the biofilm-forming mutant, leading to synthesis and secretion of EbfG1-4 and supporting the formation of biofilms.

FEMS7-0861
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

APPLICATION OF NANOSTRING NCOUNTER TECHNOLOGY FOR MEASURING BACTERIAL POPULATION DIVERSITY

Z.J. Seng[1], Y.Y. Cheng[1], J.K.H. Yam[1], L. Yang[1]
[1]Singapore Centre for Environmental Life Sciences Engineering SCELSE,
Singapore Centre for Environmental Life Sciences Engineering SCELSE, Singapore, Singapore

Backgrounds

Rapid measurement of microbial population diversity and dynamics remains to be a challenge for microbial ecology investigations. Classic approaches such as the cultivation-based assays and Fluorescence In Situ Hybridization (FISH) were generally low throughput. 16S rRNA gene amplicon sequencing is widely used for studying microbial population diversity, while it is known for the generation of PCR bias. The nCounter technology has been developed by NanoString to detect unique signal from complex hybridized samples at single molecular level without the need of PCR amplification.

Objectives

To customized nCounter array to measure the 16S rRNA genes of artificial microbial community.

Methods

Total RNAs of different bacterial species has been mixed together by different proportions in three different groups before nCounter analysis. Our results showed that the 16S rRNA read detected from each bacterial species by nCounter analysis correlates well with each species' actual total RNA added to the pool during grouping. The variation in the percentage analysis might due to the fact the total RNA from each bacterial species contains different amounts of 16S rRNA after extraction.

Conclusions

In summary, we conclude that the nCounter technology will be a useful tool for rapid and sensitive analysis of microbial diversity and dynamics based on the 16S rRNA gene.

FEMS7-1020
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PSEUDOMONAS AERUGINOSA PSL EXOPOLYSACCHARIDE INTERACTS WITH THE ANTIMICROBIAL PEPTIDE LG21 AND ATTENUATES ITS EFFECT

S.F. Chin[1], S. Sinha[2], S. Bhattacharjya[2], L. Yang[1]
[1]Nanyang Technological University,
Singapore Centre for Environmental Life Sciences Engineering SCELSE, Singapore, Singapore
[2]Nanyang Technological University, School of Biological Science SBS, Singapore, Singapore

Backgrounds

Biofilm formation by opportunistic pathogens serves as one of the major causes of chronic and persist infections. Bacterial cells in the biofilms are embedded in their self-generated protective extracellular polymeric substances (EPS), which include exopolysaccharides, large adhesin proteins and extracellular DNA.

Objectives

In this study, we identified an antimicrobial peptide (AMPs), LG21 that is able to interact specifically with the Psl exopolysaccharide of *Pseudomonas aeruginosa*. Psl reduces the antimicrobial efficacy of LG21 against *P. aeruginosa*. Psl also attenuates the pyoverdine synthesis inhibitory effects of LG21 on the Psl-overproducing *P. aeruginosa* variants.

Methods

Confocal laser scanning microscope was used to screen for the binding of rhodamine-tagged LG21. Rhodamine-tagged LG21 that were able to bind to *PAO1* biofilms but not *ΔpslBCD* biofilms were identified and used for further characterization.

The target binding of the LG21 was further confirmed by NMR studies, via performing a series of one-dimensional H NMR experiments on crude extracted psl and pel with the AMPs.

A dose dependent study of LG21 on the pyoverdine synthesis of psl-over producing strain (Δ*wspF)* and psl knock-out *(ΔwspFΔpslBCD)* strain were performed. Δ*wspF*Δ*pslBCD* lost the capacity to counter interact with LG21 and addition of LG21 to its cultures reduced significantly more pyoverdine synthesis compared to the *ΔwspF* cultures.

Conclusions

Our study suggests that biofilm EPS components might be able to modulate the host innate immune response via interacting with the AMPs.

FEMS7-1655
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CO-CULTURING OF FUNGAL STRAINS WITH THE PHYTOPATHOGEN BOTRYTIS CINEREA INDUCES THE PRODUCTION OF CHEMICAL DIVERSITY, WITH APPLICATION AS AGROCHEMICAL AND THERAPEUTIC AGENTS

R. Serrano[1], V. González-Menéndez[1], L. Rodríguez[2], J. Martin[3], J.R. Tormo[3], O. Genilloud[1]
[1]Fundacion MEDINA, Microbiology, Granada, Spain
[2]Fundacion MEDINA, Screening, Granada, Spain
[3]Fundacion MEDINA, Chemistry, Granada, Spain

Backgrounds

Microorganisms are a potential source of secondary metabolites (SMs), but frequently they are not expressed under standard laboratory conditions. Co-culturing of fungi has proved to be an efficient tool to simulate interactions between different microorganisms that may occur in their natural environment and can induce new bioactive molecules.

Objectives

Co-culturing study on the induction of new SMs in fungal strains related to ecological niches of the phytopathogen fungus *B. cinerea*, and evaluation of the activities against a panel of pathogens and their cytotoxicity profile.

Methods

We selected *Botrytis cinerea* as a model to be confronted to a large set of fungal strains (762) by using a co-culturing methodology on agar media. A collection of extracts was obtained from the antagonistic fungal interactions zones with potential of containing new bioactive molecules. The antifungal active extracts, obtained from the fungal interaction with *B. cinerea*, was also screened against a panel of three additional important phytopathogens (*Colletotrichum acutatum*, *Fusarium proliferatum* and *Magnaporthe grisea*) and two relevant human pathogens (*Candida albicans* and *Aspergillus fumigatus*). Their cytotoxicity was also evaluated against the human hepatocellular carcinoma cell line (HepG2) and the parasitic cell line (SF9). We compared by LC-HRMS the chemical profiles of 93 relevant co-culture antifungal extracts with their corresponding axenic control extracts and identified a wide variety of induced molecules including both, known and novel compounds.

Conclusions

Fungal co-culturing has permitted to induce different microbial interactions that generated new chemical diversity, setting up a methodology with wide potential both in drug discovery and crop protection.

FEMS7-1639
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

QUORUM QUENCHING BACTERIA ISOLATED FROM MBR PLANTS TREATING LEACHATE FROM SOLID RESIDUES IN SPAIN

S. Serrano[1], A. Soler[1], L. Arregui[1], D. Marquina[1], C. Álvarez-Requena[2], F. Muñagorri[2], C. García-Vera[2], A. Santos[1]
[1]Universidad Complutense de Madrid, Microbiología III, Madrid, Spain
[2]URBASER S.A., Área de Proyectos I+D+i, Madrid, Spain

Backgrounds

Quorum sensing (QS) is a mechanism dependent on bacterial density. This coordinated process is mediated by the synthesis and secretion of signal molecules called autoinducers (AIs). *N*-acyl-homoserine lactones (AHLs) are the most common AIs used by Gram-negative bacteria and are involved in biofilm formation. Quorum quenching (QQ) is the interference of QS by producing hydrolyzing enzymes, among others strategies.

Objectives

The main objective of the present study is to identify QS and QQ strains from MBR Wastewater Treatment Plants.

Methods

Plausible biofouling strains have been determined by their adhesion capability (Crystal Violet method) using AHL biosensor strains (*Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NT1). A total of 91 strains had been isolated from two MBR plants treating solid residues. Twenty isolates of Gram-negative strains were tested, four of them were classified as strongly adherent and one as moderately adherent. The QQ potential of 71 isolates of Gram-positive strains has been studied, showing that 15 of them could reduce at least one AHL activity. The analysis of 16S rRNA gene sequence showed the relevance of the genus *Bacillus* in the disruption of QS mechanism and the relevance of the *Pseudomonas* and *Aeromonas* genera in the production of biofilms.

Conclusions

The studies about the ability of QQ-bacteria in MBR systems intended to treat leachate from municipal solid waste are scarce, so this study could contribute to solve biofouling problems, in order to increase the useful lifespan of the membranes, minimizing operational costs.

FEMS7-0476
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MULTI-DRUG RESISTANT ACINETOBACTER BAUMANNII FROM HOSPITAL WASTEWATER OF ZAGREB, CROATIA

M. Seruga Music[1], J. Hrenovic[1], D. Skoric[1], I. Goic-Barisic[2], B. Hunjak[3], S. Kazazic[4], T. Ivankovic[1]
[1]University of Zagreb, Department of Biology, Zagreb, Croatia
[2]University Hospital Centre Split and University of Split School of Medicine, Department of Clinical Microbiology, Split, Croatia
[3]Croatian Institute of Public Health, Croatian Institute of Public Health, Zagreb, Croatia
[4]Ruđer Boskovic Institute, Division of Physical Chemistry, Zagreb, Croatia

Backgrounds

Acinetobacter baumannii is well known emerging hospital pathogen of the 21st century. Multi-drug resistant (MDR) *A. baumannii* are found in untreated as well as in chemically treated hospital wastewaters. However, there is no clear evidence about their origin.

Objectives

The aim of this study was to screen the hospital wastewater for the presence of viable *A. baumannii* and to find the correlation between recovered wastewater and clinical isolates.

Methods

The sampling of the hospital wastewater was done at the central manhole of the Special Hospital for Pulmonary Diseases in Zagreb, Croatia. The clinical isolate was recovered on the exact date from bronchial aspirate of patient hospital. The isolation of *A. baumannii* was performed on CHROMagar Acinetobacter supplemented after incubation at 42°C/48h.

One clinical and six isolates from hospital wastewater gave reliable MALDI-TOF MS score values ranging from 2.021-2.271 confirming the identity of *A. baumannii* colonies. All isolates were MDR and shared the resistance to carbapenems and fluoroquinolones, but sensitivity to colistin. MLST analysis following Oxford scheme revealed that all isolates belong to the ST-195 clustering into the CC92 within the IC2.

Conclusions

This study confirmed the occurrence of viable MDR *A. baumannii* in hospital wastewater in Zagreb. Close relatedness of isolates from hospital wastewater with the clinical isolates from the same hospital in the period of monitoring suggests the possible origin of recovered wastewater *A. baumannii.* This finding confirms the need for proper treatment and disposal of untreated hospital wastewaters in order to prevent the spread of MDR *A. baumannii* in nature.

FEMS7-3224
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL DIVERSITY OF TWO HOT SPRINGS IN PORTUGAL

A. Polónia[1], R. Severino[1], L. Albuquerque[2], H. Froufe[1], C. Egas[1], M. da Costa[2]
[1]Biocant- Biotechnology Innovation Center, Next Generation Sequencing Unit, Cantanhede, Portugal
[2]University of Coimbra, Center for Neuroscience and Cell Biology, Coimbra, Portugal

Backgrounds

The microbial diversity of hydrothermal areas has been constrained by the lack of extensive studies of the combined examination of the culture-dependent and culture-independent methods.

Objectives

In this respect we have begun an extensive study of the biodiversity of hydrothermal areas in Portugal, which reach vent temperatures of nearly 80ºC.

Methods

The biodiversity of two hot springs at S. Pedro do Sul and Chaves were examined using culture-independent methods – metagenome sequencing and analysis; and culture-dependent methods – cultivation and 16S rRNA gene sequence analysis.

Conclusions

Metagenome analysis at S. Pedro do Sul, with water temperature of 63ºC and pH 8.9, revealed the presence of primarily *Hydrogenothermaceae*, *Nitrospirales*, *Anaerolineae*. Eighty-seven isolates were recovered at random and identified by 16S rRNA gene sequence analysis as belonging to the genus *Thermus*, *Meiothermus*, *Rubrobacter*, *Geobacillus* and *Anoxybacillus*. The hot spring at Chaves comprises two boreholes with water temperatures of 65ºC and 76ºC. Metagenome analysis of the combined boreholes had a large predominance of bacteria of the genus *Thermus*, *Thermodesulfovibrionaceae*, *Aquificaceae* and *Thermodesulfobacteriaceae*. The archaea detected were primarily *Crenarchaeota*, unassigned Archaea and a few *Euryarchaeta*. The preliminary identification of the isolates indicates that they belong to the genus *Geobacillus*, *Thermus* and *Meiothermus*. The diversity both in terms of cultivation and in situ examination of the 16S rRNA gene sequence analysis indicates large difference in the prokaryotic communities. The reason for these differences is unknown, but may be related to the levels of reduced sulfur compounds, namely hydrogen sulfide, in the water vented at Chaves.

FEMS7-0727
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ASSESSMENT OF BIO-FERTILIZER POTENTIAL OF PSEUDOMONAS AURANTIACA ISOLATES AND ANALYSIS OF THEIR ANTI-MICROBIAL METABOLITES

I. Shahid[1], M. Rizwan[2], D. Noreen Baig[1], K. Abdulla Malik[1], S. Mehnaz[1]
[1]Forman Christian College a Chartered University, Biological Science Department, Lahore, Pakistan
[2]Applied Chemistry Research Centre- Pakistan Council of Scientific and Industrial Research PCSIR- L ahore 54600- Pakistan, Applied Chemistry Research Centre, Lahore, Pakistan

Backgrounds

Green revolution introduced heavy use of chemical fertilizers and pesticides which are threatening environmental and public health. These threats and increased antibiotic resistance of pathogens pushed microbiologists to explore new microbial sources as potential antagonists.

Objectives

In this study, eight strains of *Pseudomonas aurantiaca* were evaluated *in vitro* as potential antifungals against phyto-pathogens of rice, chickpea and sugarcane.

Methods

P. aurantiaca strains were isolated from cotton, cactus and para grass and compared with reference *P. aurantiaca* PB-St2 (sugarcane) strain. Antifungal activity of these strains was determined by agar diffusion method based on inhibition of mycelial growth. On average, all strains exhibited 0.8 cm inhibition zones against the causal agents of root rot, seedling blight, red and stem rot. All secondary metabolites were isolated by the use of solvent partitioning and subjected to LC/ESI/MS for confirmation of compounds.

Conclusions

The ESI-mass spectra obtained were used to characterize the surfactants ionization behavior and $[M + H]^+$ and $[M + Na]^+$ ions were monitored for phenazines, lahorenoic acids and cyclic lipopeptide (WLIP). This study demonstrated the production of WLIP and Lahorenoic acid A for the first time from *Pseudomonas chlororaphis.* Production of extracellular enzymes such as cellulase, protease, phosphatase, and lipase were also detected. In addition, these strains were screened for indole-3-acetic acid, HCN, siderophore production and for zinc solubilization. Plant experiments were conducted on wheat and showed considerable increase in shoots and root weight. Our results support that based on their antifungal metabolites and plant growth promoting traits, these strains can be helpful as biofertilizer.

FEMS7-0371
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ARE THE EFFECTS OF BIOINOCULANTS RELATED TO THEIR PERSISTENCE IN RHIZOSPHERE?- A CASE STUDY WITH CAJANUS CAJAN

R. Sharma[1], J.S. Paliwal[1], D. Dogra[1], V.S. Bisaria[1], S. Sharma[1]
[1]Indian Institute of Technology Delhi, Department of Biochemical Engineering and Biotechnology, New Delhi, India

Backgrounds

Agricultural amendments interact with resident microbial community to exert cumulative effect on plants. It is therefore essential to assess their survivability in plant's rhizosphere before their widespread release into the field. Several methods have been attempted to track the bioinoculants, still tracking at strain level is considered tedious. One of the methods is to develop antibiotic resistant strains. These strains when monitored, must have a selective characteristic without interfering with their inherent ability to survive or colonize.

Objectives

Current study aimed to (a) determine the survivability of *Bacillus megaterium, Pseudomonas fluorescens* and *Azotobacter chroococcum* (both individually and in combination) in the rhizosphere of *Cajanus cajan* by generating rif mutants, and (b) find correlation, if any, between the inoculants' persistence in the rhizosphere and their impact on plant parameters.

Methods

Spontaneous rifampicin-resistant mutants of the bioinoculant strains were introduced in Pot experiment set in completely randomized design, with 'No inoculation' and 'Bulk soil' as controls. Five samplings points were chosen during the plant's growth.

In terms of the effects of rif mutants on root length, shoot length and dry weight, the treatments exerted positive impact when compared with control. Within different treatments, dual inoculations surpassed the result of mono-inoculations. With time the population of bioinoculants showed a gradual reduction while they could be detected until approximately two months after sowing. After 59 days of sowing no mutants were detected.

Conclusions

There was a sharp reduction in the abundance of applied bioinoculants with time. However, these bioinoculants exerted their positive impact on the plant growth parameters even at the harvest stage showing their long-term effects irrespective of their persistence.

FEMS7-0653
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DIFFERENTIAL STRESS RESPONSE OF COAL MINE ISOLATES BACILLUS SUBTILIS MNU16, ALCALIGENES FAECALIS MNU13 AND LEUCOBACTER ARIDICOLLIS MNU27 AND ITS IMPACT ON BIOREMEDIATION POTENTIAL

S. Sharma[1], *N. Upadhyay*[1], *R. Rani*[1], *D.K. Tripathi*[2]
[1]*Motilal Nehru National Institute of Technology Allahabad, Department of Biotechnology, Allahabad, India*
[2]*Motilal Nehru National Institute of Technology Allahabad, Centre for Medical Diagnostic and Research, Allahabad, India*

Backgrounds

Coal mining results in the destruction of land ecosystem by removing the aboveground vegetation and causes an increases heavy metal contaminants in soils which influences microbial activity and plant growth. The utilization of the synergistic effect of microorganisms and plants is an effective approach of bioremediation that ensures a more efficient clean-up of heavy metal contaminated soils.

Objectives

To study the adaptive changes of bacteria in response to different Cr(VI) concentrations and their potential to remediate chromium contaminated soils.

Methods

Floral diversity of the site was analyzed by quadrat analysis technique. Bacteria were screened for stress tolerance and plant growth promoting (PGP) potential. The adaptation of potential isolates at various Cr(VI) concentrations were studied by analyzing the growth kinetics and changes in surface elemental composition and morphology was studied by using SEM–EDX and TEM analysis. The toxicity of Cr(VI) at various concentrations was further studied by flow cytometry and % Cr(VI) reduction potential was evaluated.

Conclusions

The study confirms that due to the deterioration of soil quality there is decrease in plant and microbial diversity in dump soils as compared to native soils. Three potential isolates identified as *Bacillus subtilis* MNU16, *Alcaligenes faecalis* MNU13 and *Leucobacter aridicollis* MNU27 were found to be positive for multifarious PGP activity and demonstrated Cr(VI) reduction and resistance against multi-metals, high temperature, drought and various pH ranges. The stress tolerance and chromium reduction potential of the selected PGPR strains make them suitable for the efficient microbial assisted phytoremediation of contaminated soils.

FEMS7-0654
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

A NEW INSIGHT TO PLANT GROWTH PROMOTING RHIZOBACTERIA FOR BIOREMEDIATION/ BIORECLAMATION OF COAL MINE SOILS OF SONBHADRA (U.P.) INDIA

S. Sharma[1], N. Upadhyay[1], R. Rani[1], D.K. Tripathi[2]
[1]Motilal Nehru National Institute of Technology Allahabad, Department of Biotechnology, Allahabad, India
[2]Motilal Nehru National Institute of Technology Allahabad,
Centre for Medical Diagnostic and Research, Allahabad, India

Backgrounds

Heavy metals cause environmental pollution as a result of anthropogenic activities and results into a global environmental problem as it affected an extensive area of arable land worldwide. Microorganisms native to the contaminated site were found to be resistance to various stresses and possess different mechanism for remediation of polluted soils. The significance of the current study is to explore the ability of the selected plant growth promoting rhizobacteria for tolerance and reduction of hexavalent chromium.

Objectives

To screen potential chromium resistant-PGPR isolates and to evaluate its impact on hexavalent chromium reduction.

Methods

Bacterial isolate was isolated by serial dilution technique from soil samples collected from coal mining regions. They were screened for plant growth promoting (PGP) potential viz., IAA production, phosphate solubilization, siderophore production and ACC deaminase activity as per standard method. The multi-metal resistance and chromium reduction percentage of the isolates was evaluated and potential isolates were identified by 16s rRNA sequencing.

Conclusions

The study presented here leads to the delivery of potential isolates identified as *Alcaligenes faecalis* MNU13 and *Leucobacter aridicollis* MNU27 which shows significant plant growth promotion characteristics highlighting resistance to high concentration of chromium and ability to decrease the toxicity of Cr(VI) by reducing it to Cr(III) a less toxic form. These strains will be utilized for their implication of bioremediation of chromium contaminated soils. The strains along with native plant species have potential to establish a successful bioremediation strategy for maintaining a healthy ecosystem.

FEMS7-2364
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

STUDY OF BIODIVERSITY OF ENDOPHYTIC BACTERIA INHABITING THE CONIFERS AND GRAPE AS PERSPECTIVE BASE FOR MICROBIOLOGICAL PREPARATIONS

A. Shcherbakov[1], E. Shcherbakova[1], S. Mulina[1], P. Rots[1], L. Yahina[1], V. Chebotar[1]
[1]All-Russia Research Institute for Agricultural Microbiology, Laboratory of microbial technology, Saint-Petersburg- Pushkin, Russia

Backgrounds

The study of biodiversity of endophytic bacteria inhabiting seeds and seed material of woody plants (hardwood and softwood) from various ecology and geographical origin was the first explored in Russia. The study of the spatial localization of endophytic bacteria in the seeds and seed material of woody plants and the selection of cultivated forms will build a collection of strains of endophytic bacteria, study their beneficial to plants properties and select promising strains to develop microbiological preparations for agriculture.

Objectives

Cones from *Pinus sylvestris L.* and *Picea abies L.*were collected in summer 2014 at the 2 native sites in the Middle Volga: forestry Serunskoe and forestry Zelenodolsk. Cones from *Pinus sibirica L.*were collected in autumn 2014 at the 3 native sites on the Baikal Lake coast. We isolated bacterial endophytes from plants of grapevine (*Vitis vinifera* L.) of four cultivars (Fetyaska, Rkatsiteli, Black Muskat and Muskat) sampled in Astrakhan region and Krasnodar region (Russia).

Methods

Seeds were surface sterilized with 70% ethanol and sodium hypochlorite. Endophytic bacteria were isolated from plants using an original method of surface sterilization of plant samples. Surface-sterilized cuttings were cut under sterile conditions, pieces of xylem and core were plated onto R2A medium.

Conclusions

Biodiversity of endophytic bacteria inhabiting seed and plant material of woody plants from different ecological and geographical origin has been studied. Isolated and studied 79 strains of cultivated forms of endophytic bacteria. For further research selected three most promising strains of endophytic bacteria *Bacillus megaterium*, *Bacillus thuringiensis* and *Pseudomonas sp*. Microbiome of seeds and vegetative parts of conifers plants has been studied. It was studied the spatial localization of endophytic microorganisms in plants using fluorescence *in situ* hybridization (FISH). Technological parameters for cultivation of most promising strains of endophytic bacteria have been developed and developed a laboratory protocol for production of microbial preparation.

FEMS7-3165
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECTS OF FUNGICIDES ON THE YEAST-LIKE SYMBIOTES AND THEIR HOST, NILAPARVATA LUGENS STAL (HEMIPTERA: DELPHACIDAE)

X. Shentu[1], *L. Danting*[1], *Y. Xiaoping*[1]
[1]*China Jiliang University, College of Life Sciences, Hangzhou, China*

Backgrounds

Yeast-like symbiotes (YLS) are endosymbionts that are closely related to the growth, development and reproduction of their host, the brown planthopper (BPH), *Nilaparvata lugens* Stål (Hemiptera: Delphacidae).

Objectives

In order to understand the relationship between the population of YLS in BPH cells and the survival rate of BPH.

Methods

Eight different fungicides were applied to rice plants infested by BPH, and the number of YLS and mortality of BPH were determined. Three of the fungicides, 27% toyocamycin & tetramycin P & tetrin B & tetramycin A, 0.01% trichodermin, and 75% trifloxystrobin & tebuconazole WG, were found to significantly reduce the number of YLS in BPH, subsequently causing a high mortality of BPH. The three fungicides were each mixed with a commonly used insecticide-imidacloprid, and the fungicide/insecticide mixtures could cause a marked reduction in YLS number in BPH, resulting in a significantly higher mortality of BPH than did the imidacloprid alone. The mixture of 27% toyocamycin & tetramycin P & tetrin B & tetramycin A with imidacloprid showed the best inhibitory effect on BPH population.

Conclusions

Our study demonstrated a high dependence of the BPH survival rate on the number of YLS harbored in BPH fat-body cells. It implies that using specific fungicides as an additive to imidacloprid for controlling BPH could be a novel way to enhance the efficacy of insecticide, minimizing the use of imidacloprid in paddy fields.

FEMS7-1836
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

GUT MICROBIAL MODULATION BY ANTIBIOTICS IMPROVES GLUCOSE HOMEOSTASIS OF DIET-INDUCED OBESE MICE

N.R. Shin[1], J.W. Bae[1]
[1]Kyung Hee University, Department of Biology, Seoul, Republic of Korea

Backgrounds

An excessive energy intake promotes metabolic disorders such as obesity and type 2 diabetes. Increasing evidence indicates that alterations in the gut microbiota composition play a role in the development of obesity-induced diabetes. Recently, we have demonstrated that treatment of oral antibiotic regimen in diet-induced obese (DIO) mice improved glucose homeostasis.

Objectives

We hypothesized that antibiotic treatment can affect gut microbial community composition and subsequently glucose metabolism in DIO mice.

Methods

C57BL/6 mice on either a normal-chow diet or a high-fat diet were treated with carbenicillin, metronidazole, neomycin, vancomycin or all mixture. We examined the response of gut microbiota and glucose homeostasis after 8 weeks of diet intervention and antibiotic treatment for 5 weeks. The fecal microbiota composition was assessed by analysis of 16S rRNA gene sequences using 454 pyrosequencing.

Conclusions

Although an administration of oral antibiotic altered the overall gut microbial composition of both lean and obese mice, DIO mice showed more profound shift in their microbial community profiles of gut microbiota. There were differences in the changes of gut microbial community in response to each antibiotic; however, glucose homeostasis of antibiotic-treated DIO mice was significantly improved regardless of type of antibiotics. These results suggest that oral antibiotic treatment ameliorate impaired glucose metabolism of DIO mice by modulation of the gut microbiota, indicating that this modified gut microbiota may alleviate dysbiosis induced by high-fat diet. As a follow-up study, it would be precise to tracking the specific gut microbial group or single bacteria that modify glucose and energy metabolism of the host.

FEMS7-1616
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EXPRESSION OF AN ALCOHOL DEHYDROGENASE GENE IN A HETEROTROPHIC BACTERIUM INDUCES THE OLIGOTROPHIC GROWTH WITH CARBON DIOXIDE FIXATION

S. Inaba[1], *H. Kato*[1], *Y. Ohtsubo*[1], *M. Tsuda*[1], *Y. Nagata*[1]
[1]*Tohoku University Graduate School, Environmental Life Sciences, Sendai, Japan*

Backgrounds

The gamma-hexachlorocyclohexane-degrading *Sphingobium japonicum* strain UT26 is a heterotrophic bacterium that needs organic carbon source for its growth. Although UT26 cannot grow on minimal salt agar medium prepared without adding any organic carbon sources, its transposon-inserted mutants able to grow on such medium were isolated. This phenotype was designated oligotrophic growth (OG).

Objectives

The aim of this study is to investigate the details and mechanism of OG in the UT26 mutants.

Methods

These mutants also grew on the organic carbon source-free minimal salt medium that was solidified by gellan gum, and subsequent analysis indicated no assimilation of agar or gellan gum by the mutants. It was revealed that OG was accompanied with carbon dioxide fixation although UT26 does not have the genes for key enzymes of known autotrophic carbon dioxide fixation mechanisms. The transposon in the OG mutants was inserted just upstream of the putative Zn-dependent alcohol dehydrogenase (ADH) gene (*adhX*) so that the *adhX* gene was constitutively expressed by the transposon-derived promoter. The *adhX* deletion mutant did not exhibit OG phenotype, and this phenotype was observed when the deletant had a plasmid carrying the *adhX* gene under the control of constitutive promoter. The purified His-tagged AdhX protein expressed in *Escherichia coli* showed ADH activity towards methanol and other alcohols, and site-directed mutagenesis of *adhX* indicated correlation between the ADH activity and OG.

Conclusions

These results clearly showed that the constitutive expression of the AdhX protein with ADH activity in UT26 leads to OG with carbon dioxide fixation.

FEMS7-3121
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

GUT PHAGEOMICS: YET ANOTHER VIEW OF HUMAN GUT MICROBIOME

A. Shkoporov[1], A. Forde[1], K. Daly[1], S. McDonnell[1], J. Nolan[1], O. O'Regan[1], L. Draper[1], S. Stockdale[1], M. Dalmasso[1], F. Ryan[1], A. McCann[1], P. Ross[1], C. Hill[1]
[1]APC Microbiome Institute, Gut Phageomics Lab, Cork, Ireland

Backgrounds

A healthy human gut is populated by an enormously diverse and dense consortium of microorganisms. While the bacterial component of this consortium has received significant attention in the last decade, relatively little is known about viral (and in particular, the phage) component. While metagenomic sequencing provides a powerful tool for studying phage populations, little has been done to standardise or validate sequencing and bioinformatic analysis strategies, or to analyse the impact of potentially confounding factors such as storage or handling conditions.

Objectives

1) To develop and validate a protocol for metagenomic analysis of viral/bacteriophage populations in human faeces 2) To compare reproducibility and resolution of this protocol with the more traditionally used 16S rDNA amplicon sequencing methodology. 3) To analyse operator-to-operator reproducibility of the protocol, the impact of sample storage at various temperatures, the effect of repeated freeze/thaw cycles, and the addition of exogenous viral standards for absolute quantification of VLPs.

Methods

Faecal samples collected from healthy and patient cohorts were enriched for virus like particles. Viral/bacteriophage DNA was extracted and sequenced using the Illumina HiSeq platform.

Conclusions

The gut phageome sequencing method developed in this study provides an essential complementary tool to 16S amplicon sequencing for studying (and quantifying) organisms in the gut microbiome. The accuracy and reproducibility of results compare favourably between the methods and phageomics provides a valuable additional view of human gut microbiota structure and composition. Rapid storage and limited freeze-thaw cycling of faecal samples is recommended for optimum results.

FEMS7-1665
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RAPID DEGRADATION OF LIGNOCELLULOSIC RESIDUES GENERATED AT COLDER REGIONS USING SYNTROPHIC INTERACTIONS BETWEEN PSYCHROPHILES AND MESOPHILES

L. Shukla[1], A. Senapati[1], A. Suman[1]
[1]Indian Agricultural Research Institute, Division of Microbiology, New Delhi, India

Backgrounds

Sustainable management of lignocellulose residues for improving soil fertility by biodegradation into compost has been widely practised in agricultural regions across temperate regions. In extreme cold climates, degradation rate of lignocellulose to compost is lower due to the fact that at low temperature < 10°C the activity of mesophilic microorganisms slows down, resulting in delayed onset of composting.

Objectives

This study focuses on accelerating lignocellulose transformation processes by exploiting the biological aspect of composting at cold climates through syntrophic interactions between lignocellulolytic psychrophiles and mesophiles.

Methods

Lignocellulolytic microorganisms with plant beneficial activities were screened for rapid decomposition of lignocellulose using rice straw as substrate. This included psychrophilic microorganisms *Eupenicillium crustaceum*, *Paciliomycis* sp., *Bacillus atropheus, Bacillus* sp. and mesophilic microorganisms *Aspergillus awamori, Trichoderma viride, Aspergillus nidulans* and *Phanerochaete chrysosporium* respectively. Following optimization of inoculum dose and nitrogen amendments, field trials were conducted across arable regions of the Himalayan range during extreme cold environments. Post harvest residues from rice, wheat and maize crops were used as substrates. Composting was assessed through evaluating the physicochemical parameters (colour, texture, moisture level, pH, electrical conductivity, total carbon, total nitrogen, humus content and structural analysis of lignin), biochemical parameters (dehydrogenase activity, xylanase and alkaline phosphatase), microbiological parameters (viable cell count, microbial biomass carbon and N-acetyl glucosamine level) and assessing phytotoxicity through germination tests.

Conclusions

Application of farm and poultry manure as nitrogen source restricted the use of chemical nitrogen source used for adjusting C/N ratio. Composting duration of lignocellulosic residues was 60 days only.

FEMS7-0936
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

TREATMENT OF VINASSE WITH A MIXTURE OF WILD YEAST STRAINS TO REDUCE SOIL AND FRESH WATER POLLUTION

C. Silva[1], J. Pires[1], G. Ferreira[2], K. Reis[2], R. Schwan[2]
[1]Federal University of Lavras, Biology/ Microbiology, Lavras, Brazil
[2]Federal University of Lavras, Biology/Microbiology, Lavras, Brazil

Backgrounds

The vinasse is a waterwaste from cachaça, tequila or biofuel production. Vinasse show high content pollution and needs a treatment before to be launched in the environment.

Objectives

This study evaluated the use of vinasse as a substrate for microbial biomass production and its disposal impact on the environment. .

Methods

Two *Saccharomyces cerevisiae* strains, CCMA 0137 and CCMA 0188, were grown in fresh and treated vinasse. Biomass productivity, physicochemical properties, nutritional composition, and anti-nutritional factors were evaluated. Spent vinasse was analysed and evaluated in terms of phytotoxicity and toxicity on soil microbiota in the short (5 days) and medium (60 days) term.

Conclusions

The microbial biomass showed high levels of essential amino acids (3.78%), varying levels of chemical elements, and low nucleic acid content (2.38%). Following biological treatment, vinasse biochemical oxygen demand (BOD) and chemical oxygen demand (COD) decreased to 51.56 and 29.29%, respectively. Cultivation with *S. cerevisiae* significantly reduced short term phytotoxicity and toxicity of spent vinasse. Prokaryotic microorganisms were the most sensitive to vinasse application, suggesting their possible use as biomarkers for monitoring the impact of this effluent on soil.

Financial Support: CNPq, FAPEMIG, CAPES

FEMS7-2120
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE GENOME OF THE BACTERIAL ENDOSYMBIONT OF THE HETEROPTERAN HENESTARIS HALOPHILUS REVEALS THE REASONS OF THIS SYMBIOSIS AND SERVES TO DISCUSS TAXONOMIC AFFILIATIONS IN SYMBIONTS

F.J. Silva[1], D. Santos-Garcia[2], S. Morin[2], S.M. Kuechler[3]
[1]Universitat de València,
Institute for Integrative Systems Biology and Institut Cavanilles de Biodiversitat i Biol. Evolutiva, Paterna, Spain
[2]The Hebrew University of Jerusalem, Department of Entomology, Rehovot, Israel
[3]University of Bayreuth, Department of Animal Ecology II, Bayreuth, Germany

Backgrounds

The advances of genomics have allowed the characterization of many unculturable species such as the bacterial endosymbionts of insects. Most of these associations started many million years ago and helped to the diversification of many host lineages. The characterization of the endosymbiont gene repertoire has allowed, in many cases, determining the reasons for such successful associations. However, the taxonomic affiliations given to many of these symbionts follow heterogeneous criteria. The use of phylogenomics and genomic similarity indices may help to arrive to consensus criteria for the assignment of new species and genus names.

Objectives

The aim of this work was the characterization of the genome of the gamma-proteobacterial endosymbiont of the lygaeoid bug *Henestaris halophilus* and the determination of the reasons for the start and persistence of this symbiotic relationship.

Methods

DNA from purified bacteriomes was sequenced with Illumina HiSeq paired-ends. Several computational methods were used to produce the assembly, annotation and analysis of the genome. Phylogenomics, average nucleotide (ANI) and amino acid (AAI) identity were used for taxonomic assignment.

Conclusions

The insect-bacterial association was not recent since its genome shows several characteristics of intermediate to long reductive evolution (1.62 Mb genome, 713 CDS, no mobile elements, etc.). The main reason for the persistence of this relationship has been the ability of the symbiont to complement the deficient insect diet composed by seeds/vascular tissue of some halophytes. We propose for this new symbiont the name *Sodalis baculum* and suggest that other *Sodalis* allied species should be assigned to the genus *Sodalis* as well.

FEMS7-2319
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE ORAL MICROBIOME IN LUNG TRANSPLANT RECIPIENTS

A. Simon-Soro[1], A. Bailey[2], M. Brown[1], I. Imai[1], V.R. Knecht[1], K. Bittinger[2], E. Clarke[2], E. Cantu[1], J.M. Diamond[1], J.D. Christie[1], F.D. Bushman[2], R.G. Collman[1]
[1]University of Pennsylvania, Department of Medicine. Perelman School of Medicine, Philadelphia, USA
[2]University of Pennsylvania, Department of Microbiology. Perelman School of Medicine, Philadelphia, USA

Backgrounds

The oral microbiota is associated with oral health, and can also influence pulmonary infections, which are an important problem in lung transplant subjects.

Objectives

The goal is to understand the bacterial and fungal communities in the oral microbiome of lung transplant patients in comparison to healthy and pre-transplant populations, and the impact of immunosuppression and other treatment and disease-related factors.

Methods

We studied 34 patients longitudinally who underwent lung transplantation. We characterized changes in oral microbiome from 6 weeks through 6 months after transplant surgery by high-throughput sequencing of bacterial 16S and fungal ITS rRNA genes in oropharyngeal samples. We included 12 healthy subjects and 23 subjects with end stage lung disease awaiting transplant as controls. Clinical data were analyzed to understand whether specific clinical parameters are related to a particular structure of the oral microbiome.

Conclusions

Our results showed that the oral microbiota of lung transplant patients have lower diversity than healthy individuals in both fungal and bacterial populations. We found that diversity decreased though time post-transplant. Those on more immunosuppressive agents showed had higher proportion of genera *Haemophilus*, *Lactobacillus*, *Rothia*, *Veillonella*, *Staphylococcus* and *Neisseria*. *Candida* spp. was enriched post-transplant. In conclusion, lung transplant patients have a distinctive oral microbiome during the 6 week to 6 month period after transplant in comparison to healthy subjects. This suggests a specific environment could be conditioned by the medication favoring the dominance of some species, displacing the wide normal oral microbiota.

FEMS7-0386
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

NEW THERMOPHILIC CHEMOLITHOAUTOTROPHIC SULFITE-REDUCING BACTERIA

G. Slobodkina[1], *E. Bonch-Osmolovskaya*[1], *A. Slobodkin*[1]
[1]*Winogradsky Institute of Microbiology,*
Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

Backgrounds

The dissimilatory metabolism of sulfur compounds is likely to have been among the earliest energy-yielding processes to sustain life. Prokaryotes capable of dissimilatory sulfite reduction are involved in global cycling of sulfur and carbon in modern anaerobic environments.

Objectives

We have isolated two strains of thermophilic anaerobic sulfite-reducing bacteria from a shallow submarine (SH388^T) and a terrestrial (SF97^T) hydrothermal vents located at Kuril Islands, Russia.

Methods

The very few simple inorganic compounds of direct volcanic origin could sustain development of these microorganisms. Both strains grew chemolithoautotrophically with molecular hydrogen as an electron donor, sodium sulfite or SO_2 gas as an electron acceptor and bicarbonate/CO_2 as a carbon source. Strain SH388^T was also able to grow by disproportionation of sulfite, SO_2 gas and elemental sulfur. Cells of the strain SH388^T were Gram-negative motile short rods. They grew optimally at 50^{o}C, pH 6.0-6.5 and NaCl concentrations 2.0-2.5% (w/v). The strain represents a novel species of a new genus within the class *Deltaproteobacteria*, for which we proposed the name *Dissulfurirhabdus thermomarina* gen. nov., sp. nov. Cells of strain SF97^T were Gram-positive motile rods. The optimal growth conditions for the novel isolate were 65^{o}C and pH 6.0-6.5. Strain SF97^T represents a novel species of a new genus in the family *Thermoanaerobacteraceae*, for which the name *Thermodesulfitimonas autotrophica* gen. nov., sp. nov. is proposed.

Conclusions

Dissulfurirhabdus thermomarina SH388^T is a first thermophilic disproportionator of sulfur compounds isolated from a shallow sea environment. *Thermodesulfitimonas autotrophica* SF97^T is a first obligate sulfite-reducer.

FEMS7-2148
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

USE OF BDELLOVIBRIO AND LIKE ORGANISMS (BALOS) IN PATHOGEN CONTROL AND BIOREMEDIATION: QUANTIFICATION OF ITS PREDATORY CAPACITY UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

R. Solana-Guillén[1], *F. Torrella*[1]
[1]*University of Murcia, Genetics and Microbiology, Murcia, Spain*

Backgrounds

The frequent occurrence of antibiotic multi-resistant pathogenic Gram-negative bacteria causing human and animal infections has aroused interest in the predatory capacity of BALOs as control agents. The BALOs' growth dynamics under "classical in vitro cultivation" is well known, but the real micro-environments where the predatory activity of these organisms takes place are quite different and diverse.

Objectives

The purpose of this study has been to know more about the predatory capacity of various strains of BALOs in laboratory microcosms with different suspension media ("natural water" *vs* NB-10 medium), while preying on either single species or on mixed prey bacterial suspensions.

Methods

Wild type BALOs were isolated from urban wastewaters (lysis plaques, double layer NB-10-agar). Primary isolations were made using *K.pneumoniae, E.coli*, *S. typhimurium* and other Gram-negatives as prey. Each BALO was purified, maintained and cultured in the prey strain in which was first obtained until used to check its predatory capacity on other taxa.

Conclusions

Quantitative predatory tests ("quantified host range") showed differences in the predatory dynamics and final yield of BALOs, particularly a strain isolated on *K. pneumoniae* did best in their isolation host than when preying on other susceptible enterobacteriaceae. That behavior was observed both in "natural water medium" and also in NB-10. In mixed suspensions of different susceptible preys, a specific BALO lysed preferentially cells of the species in which it was isolated originally from wastewater. These results show that in order to use BALOs as bacterial control agents, a careful strain selection is necessary.

FEMS7-0033
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ROLE OF TEMPERATURE INCREASE ON BACTERIAL CARBON FLUX TOWARDS HIGHER TROPHIC LEVELS IN THE ADRIATIC SEA

M. Solic[1], *D. Santic*[1], *S. Sestanovic*[1]
[1]*Institute of Oceanography and Fisheries, Laboratory of Microbiology, Split, Croatia*

Backgrounds

Mediterranean Sea (including Adriatic Sea) has been identified as a 'hotspot' for climate change, with prediction of the increase in water temperature of 2-4^{o}C over next few decades. Being mainly oligotrophic, and strongly phosphorus limited, Adriatic Sea is characterised by important role of microbial food web in production and transfer of biomass and energy towards higher trophic levels.

Objectives

We hypothesized that predicted rise in temperature of 3^{o}C in the near future may be resulted with increase of bacterial production and bacterial losses to grazers which could significantly enlarged trophic base for metazoans.

Methods

This empirical study is based on combined 'space-for-time-substitution' analysis and experimental approach (*in situ* grazing experiments performed). 'Space-for-time-substitution' analysis includes 3583 data sets of seawater temperature, salinity, inorganic nutrients (P, N, Si), bacterial abundance, HNF abundance and bacterial production, collected at 75 sampling sites located all over the central and south Adriatic Sea during the 2014-2016 period. Grazing on bacteria and bacterial carbon flux towards higher trophic levels were studied by 36 *in situ* experiments, which were performed on the monthly bases at three sites in the central Adriatic, covering temperature range from 10.26^{o}C to 25.07^{o}C.

Conclusions

Results showed that predicted temperature increase of 3^{o}C in the near future, as a result of global warming, could cause a significant increase in bacterial growth at temperatures lower than 16^{o} C (during the colder winter-spring period, as well as in the deeper layers). Furthermore, temperature increase of 3^{o}C, could double the grazing on bacteria by their predators, and could increase the proportion of bacterial production transferred to metazoan food web by 42%. Therefore, it is expected that global warming may further strengthen the role of microbial food web in a carbon cycle in the Adriatic Sea.

FEMS7-0377
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION OF SOIL BACTERIA WITH ANTIMICROBIAL ACTIVITIES AGAINST SEVERAL HUMAN SKIN PATHOGENS

H.G. Song[1], D.S. Lee[1]
[1]Kangwon National University, Dept. of Biological Sciences, Chuncheon, Republic of Korea

Backgrounds

At present chemical preservatives have been widely used in food and cosmetic industries, but due to their toxicity and other side-effects they should be replace to safe ones. Recently there have been many studies on development of effective and unharmful antiseptics from natural substances of plants and microorganisms.

Objectives

This study was carried out to evaluate effects of antimicrobial substances produced by isolated bacteria from various soils of South Korea, which can inhibit some skin pathogens.

Methods

Methods: Among several hundreds of bacterial strains isolated, *Paenibacillus elgii* DS381, *Bacillus subtilis* DS660 and *Paenibacillus peoriae* DS842 showed high antimicrobial activities against human skin pathogens such as *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. Isolated strains inhibited growth of all six pathogens tested. DS381, DS660 and DS842 showed 400, 800 and 200 AU/ml of maximum antimicrobial activity. When antimicrobial mechanisms were examined, strains DS660 and DS842 produced 0.056 and 0.17 mmol/ml of siderophore, respectively, and strain DS381 showed 1.56 U/ml of chitinase activity. Strains DS660 and DS842 also exhibited 167.29 and 357.28 nmol/min/mg of β-1,3 glucanase activity, respectively. In addition, oil spreading test and TLC of ethyl acetate extract of strains DS381, DS660 and DS842 culture supernatant suggested production of biosurfactants such as lipopeptide and glycolipid. Culture supernatants of strains DS381, DS660 and DS842 could reduce surface tension to 41, 33.5 and 33.5 mN/m. Antimicrobial substance of all 3 strains were stable at broad range of temperature (-22~121℃), pH (3~12) and chemical compounds (urea, Triton X-100, Tween 20 and 80).

Conclusions

These results suggest that *Paenibacillus elgii* DS381, *P. peoriae* DS842 and *Bacillus subtilis* DS660 may be utilized as an environment-friendly biocontrol agent against some human skin pathogens for commercial products such as cosmetics.

FEMS7-0809
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION OF SOME BACTERIA WITH CAPABILITIES OF DEGRADATION AND PRODUCTION INHIBITION OF AFLATOXIN B1

H.G. Song[1], *H.E. Lee*[1]
[1]*Kangwon National University, Dept. of Biological Sciences, Chuncheon, Republic of Korea*

Backgrounds

Various mycotoxins are produced by many fungi and aflatoxin B_1 (AFB_1) produced mainly by *Aspergillus flavus* is the most well-known and can be found from contaminated cereals. It is extremely toxic and carcinogenic, and poses a severe threat to animal health and brings about huge economic losses. Therefore, production of AFB_1 by *A. flavus* has to be inhibited and AFB_1 already produced should be removed to prevent economic losses from contamination of crops.

Objectives

This study investigated AFB_1 biodegradation ability of some isolated bacteria. We also tested growth inhibition and AFB_1 produced of *A. flavus* (KACC 44986).

Methods

Over 100 bacterial strains were isolated from various sources including animal feces on coumarin medium. Among them, AF34 strain degraded 72.7 and 88.44% of 0.1 and 1.25 mg/L of AFB_1, respectively in liquid culture in 72 h at 30℃. At 37℃, AF34 strain reduced 98.03 and 90.89% of 0.1 and 1.25 mg/L of AFB_1, respectively. At same conditions, strains AF38 and AF40 showed 98.08 and 83.57% of AFB_1 degradation, respectively. Then, inhibition of AFB_1 production by *A. flavus* was examined in yeast extract sucrose medium with simultaneous inoculation of isolated strains or addition of culture supernatant. The control fungal culture showed 11.91 mg/L of AFB_1 production, but the fungal culture with the addition of culture supernatant of AF34, AF38 and AF40 formed 9.76, 10.42 and 7.37 mg/L of AFB1, respectively after 120 h. Even co-inoculation of isolated bacteria reduced them to 6.32, 7.59 and 0.39 mg/L, respectively.

Conclusions

These results suggest these bacteria can be utilized to inhibit and remove of AFB_1 production from valuable resources including various crops.

FEMS7-1509
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EVOLUTION OF THE MARSUPIAL GUT MICROBIOME AND ADAPTATION TO PLANT TOXINS

R. Soo[1], M. Shiffman[1], D. Wood[1], P. Dennis[2], V. Mazumdar[1], M. Morrison[3], G. Tyson[1], P. Hugenholtz[1]
[1]Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, Brisbane, Australia
[2]School of Agriculture and Food Sciences, The University of Queensland, Brisbane, Australia
[3]The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, Australia

Backgrounds

Culture-independent methods have been applied extensively to the gut microbiomes of mammals (in particular humans and mice). Given their evolutionary and geographic novelty, surprisingly little culture-independent work has been done with iconic Australian fauna, including the diprotodont (two lower incisors) marsupials (order *Diprotodontia*). Members of this order are particularly interesting, as several are capable of anaerobically digesting eucalyptus leaves which are nutrient poor, high in lignified fibre, and enriched in natural toxins. Gut microbiota are thought to be an integral component of the toxic folivores' ability to digest eucalyptus leaves together with anatomical adaptations.

Objectives

Our objectives were 1) to survey diprotodont marsupial faecal microbiomes using 16S amplicon community profiling and shallow metagenomes and 2) recover population genomes using coverage binning of metagenomic datasets.

Methods

We collected faecal pellets from a range of marsupials that were eucalyptus eaters (possums, suborder *Phalangeriformes* and koalas, family *Phascolarctidae*) and non-eucalyptus eaters (kangaroos, family *Macropodidae* and wombats, family *Vombatidae*) from six different zoos and from the wild. We performed both amplicon and whole metagenome shotgun sequencing on 95 samples and conducted gene- and genome-centric analyses.

Conclusions

We found that faecal communities correlated with both host phylogeny and diet and identified indicator species of eucalyptus digestion.

FEMS7-1293
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IN VITRO ACTIVITY OF ENTEROCIN AP-CECT7121 AGAINST MULTI-RESISTANT STAPHYLOCOCCUS AUREUS BIOFILMS

G. Delpech[1], M. Sparo[1], M. Bistoletti[1], S. Lissarrague[1], M. Cherjovsky[1], B. Baldaccini[1], L. García Allende[1]
[1]School of Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires, Olavarría, Argentina

Backgrounds

The pathogen *Staphylococcus aureus* is able to persist on biotic and abiotic surfaces through biofilm formation. Enterocin AP-CECT7121 has shown homogenous bactericidal activity *in vitro* against *Staph. aureus* of food and clinical origin.

Objectives

Assess the eradication of biofilm-associated cells of multi-resistant clinical *Staph. aureus*.

Methods

7 multi-resistant *Staph. aureus* (HSSA32-HSSA38) isolated from blood cultures of inpatients, from Hospital Ramón Santamarina (Tandil City, Argentina) during the period January-November 2016 were studied. All included patients had hip prosthesis infections. Purified AP-CECT7121 was used. MICs determinations were done by the micro-liquid dilution method. For bactericidal activity against planktonic cells, AP-CECT7121 was added to staphylococcal cultures (10^8 CFU/mL), in a 96-well plate, to final concentrations of 1xMIC and 4xMIC, and incubated at 37°C. CFU counts were performed at 0, 4, 8, and 24 h. For assessing bactericidal activity against *Staph. aureus* biofilm cells, staphylococcal suspensions in Brain Heart Infusion broth-1% glucose were added to the wells and incubated for 24 h at 37°C. Formed biofilms were incubated with the enterocin (1XMIC and 4XMIC) for 1 and 24 h. After, CFU counts of the biofilms were done.

Conclusions

Bactericidal activity of AP-CECT7121 against planktonic cell was detected between 1- 4 h of incubation. A dose-dependent effect of AP-CECT7121 against staphylococcal biofilms was observed. AP-CECT7121 (4XMIC) showed bactericidal activity against biofilm cells, but complete killing was not achieved. AP-CECT7121 showed a significant efficacy against staphylococcal biofilms and constitutes a candidate for future applications in the prevention and treatment of biofilm-associated infections.

FEMS7-1796
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHEMICAL AND MOLECULAR BIOLOGY FINGERPRINTING OF A COMPLEX CONTAMINATED INDUSTRIAL AREA

T. Stella[1], A. Franzetti[1], I. Pietrini[2], F. de Ferra[3], G. Carpani[3], M. Marchesi[2], L. Alberti[2], D. Antonelli[4]
[1]University of Milano-Bicocca, Department of Earth and Environmental Sciences DISAT, Milan, Italy
[2]Politecnico di Milano, Department of Civil and Environmental Engineering, Milano, Italy
[3]Research Center for Non-Conventional Energy, Istituto Eni Donegani Environmental Technologies, San Donato Milanese, Italy
[4]Eni, Syndial servizi ambientali S.p.A., San Donato Milanese, Italy

Backgrounds

The management and the remediation of large contaminated areas with multiple pollutant sources and different environmental conditions represent a big challenge to site owners. Therefore, a detailed chemical and microbiological site characterization (fingerprinting) is crucial to evaluate, at first, the intrinsic remediation of the contaminated area (natural attenuation) and, then the potential of enhancing specific biodegradation processes (biostimulation).

Objectives

This study aimed at gathering chemical and molecular biology data over multiple campaigns at a contaminated industrial area to quantify the complex mixtures of contaminants, to assess the presence of potential degraders and, thus to enhance the on-going biodegradation processes.

Methods

Contaminated groundwater was collected from 30 piezometers in a restricted area of the site. Chemical analyses of chlorinated ethenes, 1,2-dichloroethane, benzene, toluene, xylene isomers, ethylbenzene and chlorinated benzenes were performed following the standard protocols. The structure of the microbial community was determined by Illumina High Throughput Sequencing, whereas its functional profile was assessed by quantitative PCR of key genes encoding for enzymes involved in specific metabolisms.

Conclusions

Vinyl chloride (VC) and 1,2-dichloroethane (1,2-DCA) were found in most of the water samples at high concentration as well as tetra- and tri-chlorinated ethenes. Illumina sequencing data showed a great bacterial diversity probably due to contamination heterogeneity. However, species belonging to *Burkholderiales* and *Rhodocyclales* orders were predominant in 1,2-DCA and VC-contaminated groundwater, respectively. The functional characterization based on the quantification of catabolic genes enconding for reductive dehalogenases (*PceA*, *TceA, VcrA, BvcA*) and oxidative enzymes (*etnC*, *etnE*) will be accomplished (on-going analysis).

FEMS7-1824
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RATES AND MECHANISMS OF ORGANOPHOSPHORUS PESTICIDE BIODEGRADATION ON GLACIERS

T. Stella[1], C. Ferrario[1], F. Pittino[1], I. Tagliaferri[1], I. Gandolfi[1], G. Bestetti[1], R.S. Azzoni[2], G. Diolaiuti[2], A. Franzetti[1], R. Ambrosini[1], S. Villa[1]
[1]University of Milano-Bicocca, Department of Earth and Environmental Sciences DISAT, Milan, Italy
[2]University of Milan, Department of Earth Sciences, Milano, Italy

Backgrounds

Organophosphorus pesticides (OPs) deposited on glacier snow and ice can undergo different partition and degradation processes, which determine their environmental fate and accumulation into ice bodies. Because of the global warming, their release in the glacial stream is growing and it contributes to their input in downstream waters. Microorganisms inhabiting the supraglacial sediments can play a pivotal role in the removal of OPs. However, the biodegradation has been neglected so far.

Objectives

This study aimed at assessing the relevance of OP biodegradative processes in cryoconite holes, a deposit of fine-grained material of atmospheric derivation laid on the bottom of small depressions of the glacier surface. In particular, our attention focused on the degradation of chlorpyrifos, an OP that can undergo both abiotic and biotic degradation.

Methods

In situ microcosm experiments were set up to simulate the cryoconite hole environment and kept under both light and dark conditions in order to estimate light influence in both biotic and abiotic degradation. GC-MS analyses were performed to evaluate the degradation of chlorpyrifos. The structure and the functions of the microbial community were determined by High Throughput Sequencing of 16S rRNA gene and of Whole Metagenomes of cryoconite.

Conclusions

Results showed that biodegradation is the most efficient process contributing to the removal of chlorpyrifos on glacier surface. Bacterial communities of cryoconitic holes involved in this process act as “biofilters” accumulating and promoting the biodegradation of chlorpyrifos. Sequencing results suggested that photoheterotrophic bacteria belonging to *Burkholderiales* might be responsible of chlorpyrifos biodegradation in cryoconite.

FEMS7-3065
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IMPACT OF LONG-TERM FERTILIZATION ON SOIL MICROBIOM

H. Stiborova[1], *M. Kracmarova*[1], *K. Demnerova*[1]
[1]*University of Chemistry and Technology, Biochemistry and Microbiology, Prague, Czech Republic*

Backgrounds

Arable soils are usually fertilized by organic or mineral fertilizers to enhance the nutrient contend desired for crop productivity. Organic fertilizers (sewage sludge, farmyard manure) are mainly used due to their high contend of organic matter and others nutrients. Compared to them, mineral fertilizers are popular for their exact composition of nutrients that could be changed as needed. The knowledge about the long-term effect of organic or mineral fertilizers on soil microbial community structure is limited.

Objectives

The changes in soil microbiome were detected in four experimental sites established in 1996. On each site is simulated common agricultural practise: potatoes, winter wheat and spring barley in three-year rotation. Simultaneously, the fields are divided into 5 individual plots and treated as follows: i) NPK mineral fertilization (doses of N-P-K nutrients were 330-90-330 kg/ha); ii) sewage sludge (330 kg N/ha); iii) sewage sludge (990 kg N/ha); iv) farmyard manure (330 kg N/ha) and v) untreated control.

Methods

From soils amended by different fertilizers was isolated DNA followed by *16S rRNA* gene amplification (V4 and V5 region) and sequencing with Illumina Miseq method. The diversity of bacterial community structure in fields was evaluated by traditional diversity indices and multivariate statistical analyses was used to link the community structure with different environmental variables.

Conclusions

Agricultural soils ammended by organic and inorganic fertilizers harbour distinct microbiomes. The response of microbial diversity mainly depends on the type and amount of organic fertilizer.

Acknowledgement:

The project was funded by the Czech Science Foundation projects no. 16-07441S.

FEMS7-1764
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EXPLORING MICROBIAL DIVERSITY BY PARALLEL CULTIVATION: INFLUENCE OF TEMPERATURE ON THE ENRICHMENT OF BIOPOLYMER PRODUCING MIXED MICROBIAL CULTURES

G. Stouten[1], C. Hogendoorn[1], S. Douwenga[1], E. Kilias[2], G. Muijzer[2], M. van Loosdrecht[1], R. Kleerebezem[1]
[1]Delft University of Technology, Environmental Biotechnology, Delft, The Netherlands
[2]University of Amsterdam, Aquatic Microbiology, Amsterdam, The Netherlands

Backgrounds

Microbial enrichments are elemental to studying ecology from both a microbial and functional point of view. Limitations in our understanding of microbial competition and coexistence can be overcome by more rigorous efforts to align functional and microbial development upon exposure of a microbial community to specific selective conditions.

Objectives

In this work we have used pulse-fed sequencing batch reactors to enrich for microbes with the highest substrate uptake rate. Two distinct ecological strategies contribute to the substrate uptake rate. Growers directly produce catalytic biomass from the substrate. Hoarders store the substrate as an internal storage compound. Stored compounds are later metabolized to catalytic biomass. This work aims for identifying the environmental conditions that favor hoarders over growers.

Methods

To this end, we have used a parallel bioreactor setup to compare functional and microbial development of a microbial community exposed to a pulse-feeding regime at different temperatures (20 to 40°C), in duplicate. High-resolution online measurements are used to identify and characterize shifts in functional process performance of the microbial community. This data is correlated with microbial dynamics, assessed by means of Illumina paired-end sequencing.

Conclusions

Within the temperature range of 25 to 35^{o}C microbes exhibiting the storing characteristic (hoarders) are enriched. At these temperatures biological duplicates show comparable enrichment patterns, however, the combined effect of differences in kinetic properties and initial numbers of microbes is inadequate to explain the succesion of events observed. The duplicates at 20^{o}C diverted into two distinct functional and microbial steady states. Generally, identified functional shifts precede the moment where microbial shifts can be derived from sequencing data. On the other hand, shifts in microbial composition did not always result in pronounced differences in observed functionality. Therefore a strong forward relation exists between observed shifts in functionality and shifts in microbial community structure.

FEMS7-1901
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIOTIC ASPECTS INVOLVED IN THE CONTROL OF SOILBORNE FUNGI DURING COMPOSTING OF VEGETABLE WASTE

F. Suárez-Estrella[1], M.J. López[1], N. Sánchez San Fulgencio[1], M.M. Jurado[1], J.A. López-González[1], J. Moreno[1]
[1]University of Almería- ceiA3, Biology and Geology. Area of Microbiology. CITE IIB., Almería, Spain

Backgrounds

High temperatures generated inside a compost pile greatly influence the eradication of plant pathogens (Ryckeboer *et al*., 2003). However, others factors should not be ruled out. In this sense, during the composting of plant wastes, an important community of thermotolerant bacteria is globally involved in the control of other phytopathogenic organisms that could be present in the plant material. The mechanisms that those bacteria use are very varied and range from substrate competition through the production of siderophores, salicylic acid, chitinase-like enzymes, to the solubilization of phosphates, as well as the release of antifungal substances such as cyanide (Mehta *et al.,* 2014).

Objectives

The main goal of this work was to confirm the hypothesis that in addition to the high temperatures reached during the composting process, the microbial community of a composting pile actively collaborates in the control of *damping-off* producing agents.

Methods

Antagonistic effect of approximately 600 bacterial isolates was assayed by dual cultures against *Pythium ultimun, Fusarium oxysporum* f.sp. *melonis, Rhizoctonia solani* and *Phytophthora capsici*. Production of siderophores, salicylic acid, cyanide, or chitinase-like enzymes was qualitatively tested.

Conclusions

Only 3% of the initial collection of isolates proved to have a multipotential character with respect to its spectrum of action against *damping-off* producing agents, as well as in relation to its capacity to produce substances of agronomic interest. Among the selected strains, the isolates belonging to the genera *Bacillus* and *Pseudomonas* were the most represented.

Mehta, C.M. et al. 2014. Waste Manag 34: 607-622

Ryckeboer, J. et al. 2003. Ann Microbiol 53 (4): 349-410

FEMS7-0637
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ACTIVE COMMUNITY IN A PARTIAL NITRITATION ANAMMOX BIOREACTOR

C. Suarez[1], F. Persson[2], M. Hermansson[1]
[1]University of Gothenburg, Department of Chemistry & Molecular Biology, Gothenburg, Sweden
[2]Chalmers University of Technology,
Division of Water Environment Technology- Department of Civil and Environmental Engineering, Gothenburg, Sweden

Backgrounds

Partial nitritation anammox (PNA) is an established technology for nitrogen removal from wastewater. Anammox bacteria and Ammonia oxidizing bacteria (AOB) are necessary for the PNA process, but are only part of a complex community in which the metabolic activity is largely unknown.

Objectives

We studied the total and active community of a mainstream PNA reactor.

Methods

The microbial community of a pilot moving bed biofilm reactor fed with real main stream wastewater was investigated. DNA and RNA were co-extracted from 3 biofilm carriers. Relative abundance of ribosomal DNA (rDNA) and ribosomal RNA (rRNA) was studied using high throughput amplicon sequencing. Active taxa are defined as those with an rRNA:rDNA ratio higher than 1.

Conclusions

Some variation was observed between different carriers in both rDNA and rRNA relative abundance of taxa. Nevertheless, for all 3 biofilms carriers, a large number of active but rare taxa were observed as well as several abundant taxa with a very low rRNA:rDNA. The anammox bacteria *Brocadia* were highly abundant in all carriers, with rRNA:rDNA close to 1. The AOB *Nitrosomonas* had an rDNA relative abundance below 1%, but were active in all carriers (rRNA:rDNA between 1.3 and 20.5). However, also other bacteria showed high activity; for instance glycogen accumulating and putatively denitrifying *Competibacter*, and non-anammox bacteria within *Planctomycetes* (*Planctomyces*) had high rRNA:rDNA. Hence, a more complex picture of the PNA communities can be obtained by comparing rDNA and rRNA relative abundance.

FEMS7-1924
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PREVALENCE OF ANTIBIOTIC RESISTANCE GENES AND POTENTIAL PATHOGENS IN STREAMBED BIOFILMS. DOES THE POLLUTION SOURCE MATTER?

J. Subirats[1], X. Triadó-Margarit[2], J.L. Balcázar[1], S. Sabater[3], C. M. Borrego[1]
[1]Catalan Institute for Water Research, Water Quality, Girona, Spain
[2]Centre d'Estudis Avançats de Blanes- CEAB-CSIC, Integrative Freshwater Ecology Group, Girona, Spain
[3]Catalan Institute for Water Research, Resources and ecosytems, Girona, Spain

Backgrounds

Wastewater discharges into surface waters impair their quality by altering not only their overall chemistry and nutrient dynamics but also by introducing antibiotic residues and antibiotic-resistant bacteria (ARB) that directly affect the streambed resistome.

Objectives

The study was aimed to assess the impact of raw and treated wastewater on streambed biofilms at three levels: *i)* on the composition of bacterial communities; *ii)* on the abundance of antibiotic resistance genes (ARGs); and *iii)* on the occurrence of wastewater-derived putative pathogens and their linkage to ARGs.

Methods

The study was carried out in two tributaries of the Ebre River that receive discharges of either treated wastewater or raw sewage. In both streams, epilithic and epipsammic biofilms were collected upstream (control) and downstream (impact) the effluent discharge. The concentration of eleven ARGs, class 1 Integron integrase (*intI1*) and 16S rRNA genes and the composition of streambed biofilms communities were determined by qPCR and high-throughput sequencing, respectively. The occurrence of potential pathogens was assessed by comparing representative OTUs against an in-house database of 283 human and animal pathogens.

Conclusions

Raw sewage discharges had a larger effect than treated wastewater on the composition of bacterial communities and their associated resistome. This effect was especially noticeable in epilithic biofilms, which showed communities more enriched in ARB and potential pathogens than epipsammic biofilms. Our study provides evidences that epilithic biofilms act as source and sink of ARGs and ARB and corroborate that they are sensitive indicators of antibiotic pollution in riverine ecosystems receiving wastewater discharges.

FEMS7-0106
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INSIGHT INTO GENOMIC PLASTICITY OF PSEUDOMONAS PUTIDA KF715 WHICH HAS UNIQUE PROPERTIES IN BIPHENYL-UTILIZING ACTIVITY AND GENOME INSTABILITY

H. Suenaga[1], H. Fujihara[2], N. Kimura[3], J. Hirose[4], T. Watanabe[5], T. Futagami[6], M. Goto[7], K. Furukawa[2]
[1]National Institute of Advanced Industrial Science and Technology AIST, Biotechnology Research Institute for Drug Discovery, Tokyo, Japan
[2]Beppu University, Department of Food and Nutrition, Beppu, Japan
[3]National Institute of Advanced Industrial Science and Technology AIST, Bioproduction Research Institute, Tsukuba, Japan
[4]University of Miyazaki, Department of Applied Chemistry- Faculty of Engineering, Miyazaki-, Japan
[5]Kyoto University, Research Institute for Sustainable Humanosphere, Uji, Japan
[6]Kagoshima University, Education and Research Center for Fermentation Studies- Faculty of Agriculture, Kagoshima, Japan
[7]Saga University, Faculty of Agriculture, Saga, Japan

Backgrounds

Biphenyl-utilizing *Pseudomonas putida* KF715 has the unique properties in both catabolic activity and genome instability. We have revealed that DNA region encoding the biphenyl metabolisms (*bph*), was frequently deleted and transferred by conjugation to closely related *P. putida* strains.

Objectives

We reveal the genome plasticity of KF715 which is comprehensive model strain for better understanding of its potential versatile catabolic properties and genome rearrangements for the environmental adaptation.

Methods

We first determined the complete nucleotide sequence of the KF715 wild type genome. Secondly, KF715 genome was compared with those of one KF715 defective mutant (KF715M2) and two transconjugants (AC30Bph+ and KT2440 Bph+) together with several *P. putida* strains available in public database.

Conclusions

The gapless KF715 genome sequence revealed large number of plasmids (pKF715A, pKF715B, pKF715C and pKF715D). Southern blot analyses indicated the majority of KF715 cell population carry the *bph* genes on chromosome and fewer carry it on plasmid pKF715A, and the pKF715A transfer by conjugation to recipient strains. These results suggested that pKF715A behave as Integrative and Conjugative Element (ICE). However, unlike to typical ICE, pKF715A carrying *bph* genes transfer by conjugation to recipient strains and did not integrate into chromosome in its recipient. Furthermore, by comparative genome analysis, a number and variety of putative genetic elements which play a significant role in genome rearrangements were found in KF715. These genome data of KF715 provide insight into the genetic plasticity and adaptability of microorganisms in various ecological niches.

FEMS7-3028
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RUMEN FERMENTATION CHARACTERISTICS AND MICROBIAL QUANTITIES OF DAIRY COWS DURING ADAPTATION PERIOD OF CHANGING DIET

L. Mamuad[1,2], S.H. Kim[2,3], Y.J. Choi[2], M. Lee[2], S.S. Lee[2]
[1]The Ohio State University, Animal Sciences, Columbus, USA
[2]Sunchon National University, Animal Science and Technology, Suncheon, Republic of Korea
[3]The Ohio State University, Animal Sciences, Wooster, USA

Backgrounds

Animals become acclimated to the treatments before data are collected during adaptation period where rumen microbiota also undergoes populational and metabolic changes.

Objectives

This study evaluated the effects of changing diet during adaptation on the rumen microbiota and its fermentation characteristics.

Methods

Three Holstein-Friesian cows were initially fed with Italian ryegrass *ad libitum* and 2 kg concentrate diet per day and for 30 days. Then, 8 kg/day of concentrate diets were given for 14 days and then changed to 2 kg/day for another 14 days. Rumen fluids were collected 2 h after morning feeding through stomach tubing at the start of the experiment, at day 7 and 14 of the diets. pH, VFA, and microbial quantities were determined.

Conclusions

The highest pH (7.02) was observed at the start of the experiment while the lowest pH (6.80) at the end of the feeding trial. Concentrations of acetate (56.76 mM), propionate (13.28 mM), and butyrate (6.60 mM) peaked ($P<0.01$) at day 14 day of consuming 8 kg concentrate. The total bacteria quantity was comparable throughout the feeding trials. *Ruminococcus flavefaciens* (4.64 and 5.08) and protozoa (1.94 and 2.00) log copies were highest at day 7 of feeding 8 kg and 2 kg concentrate diets. The lowest methanogen quantity was observed at the start of the experiment (2.96 log copies) with increasing copies until the end of the feeding trial, which was the highest (3.63 log copies). The rumen fermentation characteristics and microbial quantities changed with changing diet during adaptation period.

FEMS7-2460
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE DIFFERENTIATION OF BACTERIA INSIDE PEA SYMBIOTIC NODULES IS REGULATED BY THE PLANT GENE SYM31

A. Sulima[1], A. Afonin[1], A. Zhernakov[1], V. Zhukov[1], I. Tikhonovich[1]
[1]All-Russia Research Institute for Agricultural Microbiology ARRIAM, Laboratory for Genetics of Plant-Microbe Interaction, Saint-Petersburg, Russia

Backgrounds

The development of symbiotic nitrogen-fixing nodules in the leguminous plants (family Fabaceae) is controlled by a complex system of plant genes. Mutation analysis is a powerful tool for the study of this system. The pea (*Pisum sativum* L.) mutant Sprint-2Fix$^-$ carrying a mutation in the gene *Sym31* is characterized by a unique phenotype not described for other model legumes: halting of nodule development coupled with the block of the bacteria differentiation into a specialized symbiotic form called bacteroids.

Objectives

The aim of this study was to compare the coordinated gene expression of both symbionts in nodules of mutant and wild type plants.

Methods

To this end, the combined bacterial and plant transcriptomes of wild-type and mutant nodules, as well as that of free-living bacteria, were sequenced using the novel 5'MACE approach. This method increases the accuracy of differential gene expression analysis by sequencing only a small fragment of each mRNA directly adjacent to the 5' end.

Conclusions

Only in bacteria in wild-type nodules the increased expression of several genes related to the TCA cycle, nitrogen assimilation and transportation of nitrate was observed, while the expression of genes related to quorum sensing, chemotaxis and antibiotic synthesis was decreased. The genes responsible for metabolism of sulfite anion were active in bacteria from the wild-type nodules, while in mutant nodules their expression was significantly lower than even in free-living bacteria, which correlates with decreased expression of plant genes controlling the sulfur compounds transport.

This work was supported by Russian Science Foundation [grant 14-24-00135].

FEMS7-2069
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHARACTERIZATION AND PURIFICATION OF ANTIFUNGAL METABOLITES FROM PAENIBACILLUS SP 16A AGAINST PHYTOPATHOGENS

A. Sundararaman[1], *S.S. Lee*[1]
[1]*Kyonggi University, Life Science, Suwon, Republic of Korea*

Backgrounds

Phytophathogen *Cylindrocarpon*s sp. is detrimental to various agronomic crops, especially *Panax ginseng*. Ginseng is the most widely cultivated as medicinal herb in Korea. Ginseng field are most susceptible for plant pathogenic fungi such as *Cylindrocarpons*, *Alternaria* and *Botrytis*. After the harvest of ginseng from the farm, pathogenic fungi were latent for 3 or 4 years in the rhizosphere and they had influenced in re-cultivation of the ginseng.

Objectives

This study is to identify the potential antifungal bacteria and develop the strain as a biocontrol agent against the phytopathogen *Cylindrocarpon destructans.*

Methods

The potent candidate strain was identified as *Paenibacillus* sp. by 16S rRNA sequencing. Crude metabolite was obtained from cell free supernatant of *Paenibacillus* sp. 16a by ethyl-acetate extraction and it was dissolved in methanol for further purification by column chromatography. The fractions were obtained through the sephadex column were checked for purity by HPLC. The HPLC detections confirmed the presence of single metabolite and purified fraction was observed for antagonistic activity against all the fungal test pathogens. The radial plate assay confirmed the rate of inhibition up to 60.0% against 8 phytopathogens and the metabolite responsible for antifungal activity was identified by LC-MS to be belonging to the class of peptides.

Conclusions

The results demonstrated that *Paenibacillus* sp. 16a *in vitro* study has wide spectrum of antifungal activity to be used as a potential biocontrol agent against fungal phytopathogens. Further studies focus on the *in vivo* efficacy of strain 16a in inhibiting fungal pathogens.

FEMS7-1595
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION AND CHARACTERIZATION OF THE NOVEL SPECIES CANDIDATES FROM THE GUT OF THE SPOONBILL AND VULTURE

H. Sung[1], H.S. Kim[1], J.Y. Lee[1], W. Kang[1], P.S. Kim[1], D.W. Hyun[1], E.J. Tak[1], M.J. Jung[1], J.H. Yun[1], M.S. Kim[1], N.R. Shin[1], T.W. Whon[1], J.W. Bae[1]
[1]Kyung Hee University, Department of Biology, Seoul, Republic of Korea

Backgrounds

To investigate the bacterial community in the gut of a black-faced spoonbill (Platalea minor) and cinereous vulture (Aegypius monachus), we performed bacterial isolation from fecal samples of two birds protected by Seoul Grand Park Zoo, Korea.

Objectives

We wanted to find out the bacterial community in the gut of the birds, and discover what putative novel microbes can be recovered in a laboratory environment.

Methods

We innoculated the serially diluted fecal samples onto 5 following agars; Marine agar(MA, DifcoTM), Lactobacilli MRS agar(MRSA, DifcoTM), Tryptic soy agar(TSA, BactoTM), R2A agar(R2A, DifcoTM), MacConkey agar(Mac, BBLTM). Using this culture-dependent method, we obtained 126 isolates and 187 isolates from the fecal samples of cinereous vulture and black-faced spoonbill, respectively. Then, we conducted the phylogenetic analysis on the basis of the 16S rRNA.

Conclusions

Consequently, 29 isolates were putative novel species candidate, according to the phylogenetic analysis based on the 16S rRNA gene sequences.
At phylum level, 23, 12, and 2 strains were comprised of Firmicutes, Actinobacteria and Proteobacteria, respectively. In genus level, we found out bacteria which share high sequence similarity with the genus Tumebacillus (3), Virgibacillus (1), Sporosarcina (3), Bacillus (2), Vagococcus (2), Jeotgalibaca (1), Paenibacillus(1), Erysipelothrix(2), Actinomyces (2), Brachybacterium (3), Ornithinimicrobium (1), Kocuria (2), Herbiconiux (1), Micrococcus (1), Brevibaterium (1), Microbacterium(1), Psychrobacter (1), and Oceanisphaera (1 strain).
To identify and characterize these bacterial species, genotypic, phylogenetic, biochemical, phenotypic and chemotaxonomic analyses are in progress.

FEMS7-1441
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MONITORING BACTERIAL DEPOSITION IN POROUS MEDIA BY PREDATORY BACTERIA

R. Sungthong[1], *W. T. Sloan*[1], *A. J. Pinto*[2]
[1]*University of Glasgow, School of Engineering, Glasgow, United Kingdom*
[2]*Northeastern University, Department of Civil and Environmental Engineering, Boston- MA, USA*

Backgrounds

Bacterial deposition in porous media is an important microbial property affecting range of environmental technologies, e.g., prevention of pathogen dispersion towards groundwater, optimization of subsurface bioremediation, and development of biofiltration in water treatments.

Objectives

In order to optimize the biofiltration by exploiting predatory bacteria, we assessed how these bacteria behave through porous media of sand-packed columns and how they influence the transport and deposition of a coliform bacterium, *Escherichia coli* through the columns.

Methods

Bdellovibrio bacteriovorus HD100 and its saprophytic mutant (HI100) were utilized as predatory bacteria.

Conclusions

Our findings revealed that only ~20% of HD100 cells could deposit in the columns, and such amount of deposited HD100 cells could diminish the deposition rate of *E*. *coli* cells in the same columns by up to 35%. However, up to 55% of *E*. *coli* cells pre-deposited in the columns was removed after dosing with HD100 cell suspension. Although pre-saturated cell-free culture (CFC) broth of strain HI100 in the columns did not show significant effect on the deposition rate of *E*. *coli* cells, it could remove up to 50% of *E*. *coli* cells pre-deposited in the columns. The viability of *E*. *coli* in the column effluents did not change after dosing with HD100 cell suspension or CFC broth of strain HI100, suggesting that either deposition or detachment of *E*. *coli* cells was a result of the interfacial interactions with predatory bacteria and/or their extracellular metabolites. This study provides new insights into an attempt for implementing microbial management in the optimization of biofiltration design and performance.

FEMS7-1488
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PHYTOCHEMICAL PROPERTIES SHAPE COMMUNITY STRUCTURES OF CULTIVABLE ACTINOBACTERIA INHABITING PLANT INTERIORS OF DIFFERENT THAI PIGMENTED RICE CULTIVARS

N. Nakaew[1], R. Sungthong[2]
[1]Naresuan University, Department of Microbiology and Parasitology, Phitsanulok, Thailand
[2]University of Glasgow, School of Engineering, Glasgow, United Kingdom

Backgrounds

Microbes that live at plant interiors are known as endophytes, which are one among the best-known plant-microbe interactions. Hitherto, it is conceivable that plant interiors are a promising source of numerous bioactive actinobacteria for novel drug discovery. However, little is known about the diversity and ecological functions of cultivable actinobacteria that inhabit the plant interiors of Thai pigmented rice.

Objectives

This study aims of investigating the abundance and plant growth-promoting (PGP) potentials (e.g., antimicrobial activities and productions of ammonia, indole acetic acid, siderophores, etc.) of cultivable actinobacteria isolated from the plant interiors of different Thai pigmented rice cultivars, i.e., Hom Nin (HN) rice and Luem Pua (LP) glutinous rice.

Methods

Surface sterilized plant materials (roots, stems, and leaves) derived from 15-day-old seedlings of both rice cultivars grown in the same soil were used as the sources for isolation of actinobacteria. The water used for the final wash of the plant materials served as the controls of the surface sterilization.

Conclusions

A total number of 66 actinobacterial isolates was obtained, while both rice cultivars housed an equal isolate number (33), and more than 50% of these actinobacteria exhibited at least one PGP potential. *Streptomyces* (55%), *Microbispora* (36%), and unclassified actinobacteria (9%) were derived from HN rice, while only *Microbispora* (97%) and unclassified actinobacteria (3%) were derived from LP glutinous rice. Our findings revealed that the difference in seedling phytochemical properties of both rice cultivars would be a key factor, affecting the community structures of cultivable actinobacteria that live at the interiors of rice plants.

FEMS7-0915
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RE-APPEARANCE OF BACTERIA IN ANTIBIOTIC TREATED MICE AFTER EXPOSURE TO METHYL-MERCURY

C.S. Svanevik[1], L.S. Myrmel[2], E. Fjære[2], A. Maage[1], L. Madsen[2], B.T. Lunestad[1]
[1]National Institute of Nutrition and Seafood Research, Contaminants and Biohazards, Bergen, Norway
[2]National Institute of Nutrition and Seafood Research, Seafood in model systems, Bergen, Norway

Backgrounds

The demonstrated co-selection of resistance to antibiotics and mercury (Hg) may suggest that mercury could act as a driving force for the development of antibacterial resistance.

Objectives

This study examined the different taxa of culturable bacteria in faeces from mice treated with antibiotics and subsequently exposed to methyl mercury (MeHg).

Methods

Mice were treated with an antibiotic cocktail (AB-C) (ampicillin, vancomycin, ciprofloxacin, imipenem, and metronidazole) through the drinking water one week prior exposure of MeHg. Control groups included mice that were given no treatment, only antibiotics or only MeHg exposure. Faecal samples were examined for cultivable bacteria on selective agar plates. Heterotrophic plate counts (HPC) on Mueller-Hinton agar were examined the last week, including MH agars added three concentrations of: the AB-C, the combination of AB-C and MeHg, and only MeHg.

Conclusions

A re-appearance of Bacteroides, Enterobacteriaceae, Enterococci, and in particular Clostridiales were seen in mice treated with AB-C and exposed to MeHg.

HPC showed that faeces from mice treated with AB-C and exposed to MeHg held log 5.4 cfu/g compared to log 8.6 cfu/g in the control, and absence in mice treated with AB-C only.

These bacteria grew in all MH plates with low concentrations, and one sample grew on MH with medium concentrations of AB-C and MeHg, where the control samples did not grow.

We cannot exclude that MeHg could have triggered mechanisms in dormant bacteria to better tolerate the antibacterial treatment, and thus be the reason why the bacteria of some taxa re-appear in the mouse faeces.

FEMS7-1443
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EVALUATION OF CULTURABLE BIFIDOBACTERIAL POPULATION INHABITING THE DIGESTIVE TRACT OF WILD PIGS BASED ON FOUR MOLECULAR GENETICS METHODS

R. Pechar[1], J. Killer[1], V. Rada[1], R. Švejstil[1]
[1]Czech University of Life Sciences Prague, Department of Microbiology- Nutrition and Dietetics, Prague 6, Czech Republic

Backgrounds

One hundred forty-six fructose-6-phosphate phosphoketolase positive bacterial strains were isolated from ileal and rectal contents of twenty-one individuals of wild and feral pigs and based on PCR-DGGE technique targeting the hypervariable V3 region of the 16S rRNA genes, strains were initially differentiated into four groups: i) including probably a new *Bifidobacterium* species (86 strains), ii) including *B. boum/B. thermophilum/B. thermacidophilum* subsp. *porcinum/B. thermacidophilum* subsp. *thermacidophilum* (sub)species (49 strains), iii) represented by *Pseudoscardovia suis* (7 strains) and iv) including *B. pseudolongum* subsp. *globosum/ B. pseudolongum* subsp. *pseudolongum* (4 strains), respectively.

Objectives

The objective was to identify and differentiate intestinal bifidobacterial population of wild pig.

Methods

Strains were further identified by the 16S rRNA gene sequences, and newly using the *dnaK* (encoding the chaperone protein DnaK) and *thrS* (encoding the threonyl-tRNA synthase) gene fragments.

Conclusions

Phylogenetic study allowed to classify members of the first group into five subgroups in a separated cluster of thermophilic bifidobacteria.

Comparative and phylogenetic study based on *thrS* sequences revealed 11 STs (sequence types) classified into 5 different phylotypes, (*Bifidobacterium* sp., *B. boum*, *B. thermophilum/B. thermacidophilum* subsp. *porcinum*/*B. thermacidophilum* subsp. *thermacidophilum*, *P. suis* and *B. pseudolongum* subsp. *globosum/ B. pseudolongum* subsp. *pseudolongum*). Analyses using *dnaK* gene determined 12 STs belonging to 4 phylotypes.

Overall, above molecular genetic techniques allow to identify a probably new *Bifidobacterium* species which is predominant in the digestive tract of examined wild and feral pigs. The second largest group of culturable bifidobacteria is represented by the *B. thermophilum/B. thermacidophilum* subsp. *porcinum*/ *B. thermacidophilum* subsp. *thermacidophilum* phylotype.

FEMS7-2253
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INFERENCE OF MICROBIAL INTERACTION NETWORKS FROM MASSIVE DATA SETS THROUGH CAUSAL KNOWLEDGE DISCOVERY

J. Tackmann[1], *J.F.M. Rodrigues*[1], *C. von Mering*[1]
[1]*Universität Zürich, Institute of Molecular Life Sciences, Zürich, Switzerland*

Backgrounds

The recent explosion of metagenomic sequencing data makes tools for rapid computational analysis essential. While such software is becoming increasingly available for OTU mapping and clustering, prediction of microbial interactions based on co-occurrence is still lagging behind.

Objectives

While simple correlation-based tools scaling to large numbers of OTUs and samples exist, these do not distinguish between direct and indirect interactions, resulting in unacceptably high numbers of false positives. Approaches with better resolution, on the other hand, are so far highly limited in the size of data sets they can process. Furthermore, environmental factors, while being important modulators of microbial interactions, are usually not considered by available software. Finally, we observe traditional approaches to produce large numbers of false positives due to double-zero inflation caused by environmental niches in composite datasets.

Methods

We adopt a machine learning framework based on Probabilistic Graphical Models and inspired by causal theory to infer highly resolved microbial interactions from large data sets, with seamless integration of environmental variables and optional adjustment for sub-niches.

Conclusions

The method is highly optimised for speed, scaling to thousands of OTUs and samples and surpassing methods with comparable accuracy by more than an order of magnitude. In benchmarks on several synthetic data sets, it provides accuracy comparable to or surpassing state-of-the-art methods. We apply this approach to a massive meta-dataset of publicly available human feces samples (>37.000 samples), resulting in the largest and most diverse survey of microbial interactions in the human gut to date.

FEMS7-1580
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

VAGOCOCCUS CREMEUS SP. NOV., ISOLATED FROM THE SMALL INTESTINE OF A MARTEN, MARTES FLAVIGULA

E. Tak[1], H.S. Kim[1], J.Y. Lee[1], W. Kang[1], J.W. Bae[1]
[1]Kyung hee univrsity, Biology, seoul, Republic of Korea

Backgrounds

Martens live in wild forest and they are predator eating other wild animals like badgers, squirrels, birds, boars and elks (PARR[1] & Duckworth, 2007). It is important to investigate gut microbiota of wild animal because the gut microbiota have a lot of influence on their host (Tremaroli & Bäckhed, 2012).

Objectives

Because most wild animals including martens are protected at the state level, it is difficult to investigate their gut microbiota while they are alive. So we used a small intestinal tract sample of the marten, which was killed on the road in Pocheon-si, Gyeonggi-do, Republic of Korea and obtained from the National Institute of Biological Resources (NIBR), for investigating gut microbiota.

Methods

Homogenized intestinal tissue and fluid were diluted with filtered PBS into 10^{-5}, 10^{-6} and 10^{-7}, which were spread onto TSA, R2A, MA and, MRSA plates, respectively. After aerobic incubation at 20 ˚C and 37 ˚C, 53 isolates were obtained as a result of culture-dependent manner. Of that, strain D7T301^{T} was collected from a TSA medium at 37 ˚C.

Conclusions

Strain D7T301^{T} was isolated from the small intestinal tract sample of a *Martes flavigula*, which is Korean endemic species. The isolate is Gram-stain-positive streptococci, non-motile, facultative aerobic, catalase-negative and oxidase-negative. *Vagococcus cremeus* is proposed as a novel species within the genus *Vagococcus* on the basis of the phenotypic, phylogenetic, biochemical, chemotaxonomic and genotypic analyses, with strain D7T301^{T} as the type strain.

FEMS7-0315
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

FUNCTIONAL METAGENOMICS OF LOW LATITUDE AREAS OF THE PACIFIC OCEAN

H. Takami[1], K. Takemoto[2], W. Arai[1], Y. Ogura[3], T. Hayashi[3], K. Hamasaki[4]
[1]Japan Agency for Marine-Earth Science and Technology, Yokohama Institute, Yokohama, Japan
[2]Kyushu Insitute of Technology, Bioscience and Bioinformatics, Iizuka, Japan
[3]Kyushu University, Faculty of Medical Sciences, Fukuoka, Japan
[4]The University of Tokyo, Atmosphere and Ocean Research Institute, Kashiwa, Japan

Backgrounds

Although many metagenomic analyses of environmental samples were reported so far, functional information of microbial community is very poor.

Objectives

We performed functional metagenomics of surface sea water samples collected at 9 sites of low latitude areas in the Pacific Ocean from 2011 to 2012 to highlight difference in functional traits of ocean microbial community.

Methods

The collected samples were divided into two fractions (free-living and particle-associated ones) by different pore size filters (0.2-3.0µm and >3.0µm) and metagenomic DNAs were isolated from each sample. One to two million sequences with continuous 300 bp (paired-end) were produced by MiSeq from each sample. Amino acid sequences of the genes predicted by MetaGeneAnnotator were applied to MAPLE system for evaluation of metabolic potential in each sample.

Conclusions

According to community structure analysis based on ribosomal proteins detected by MAPLE, all free-living fractions from 9 sites were mostly composed of bacteria (>99%) although all particulate associate fractions contained achara of 3~6.5% and eukaryote of 2.4~5%. We calculated completion ratio (%) to the KEGG functional modules and the abundance of complete modules in each metagenome samples. We performed canonical correlation analysis based on the module abundance to highlight difference in functional traits between free-living and particle-associated fractions. As a result, we found that free-living fraction correlates to the modules for amino acid biosynthesis and transporters such as amino acid and metal ion. On the other hand, particulate associate fraction was found to correlate to the module of C1 unit inter conversion related to eukaryotic folate metabolism.

FEMS7-0765
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COMPARISON OF THE MICROBIOTA OF ANTERIOR AND POSTERIOR INTESTINE OF FARMED SENEGALESE SOLE (SOLEA SENEGALENSIS)

S.T. Tapia-paniagua[1], M. Domínguez Maqueda[2], I. García de la Banda[3], X. Moreno-Ventas[5], M.D.C. Balebona[2], M.A. Moriñigo[4]
[1]Universidad De Malaga, Microbiologia, Malaga, Spain
[2]Universidad De Malaga, Microbiology, Malaga, Spain
[3]Spanish Institute Of Oceanography, Estacion De Cultivos El Bocal-, Santander, Spain
[4]Universidad De Malaga, Microbiologia, Malaga, Spain
[5]Universidad de Cantabria, Santander, Spain

Backgrounds

The intestinal microbiota plays important functions in the host. In this context, previous studies have analyzed the intestinal microbiota of *Solea senegalensis*, one farmed fish with high economic interest, using conventional approaches such as DGGE. However, the use of innovative technologies, such as NGS is driving understanding of the interactions between the microbiota and the host.

Objectives

For this reason, the objective of this study was to apply NGS technology to determine and to compare the intestinal microbiota of *S. senegalensis* considering the structural and morphologic differences between anterior and posterior intestine.

Methods

Samples were collected from anterior and posterior intestine. DNA was extracted from intestinal samples and was sent to ChunLab (Seoul, Korea) to determine the microbial DNA sequences of the 16S rRNA gene by Illumina technology. Sequences were analyzed using CLcommunity™ software (ChunLab). Sequences of a length less than 200 nt were excluded from the analysis. The data were filtered for noisy sequences, checked for the presence of chimeras, and binned into OTUs at the 97% sequence similarity level. A representative sequence of each OTU was taxonomically classified. The relative abundance of microbial clades at different taxonomic levels was calculated as the average value from two independent analyses and was used to perform the comparative distribution analysis.

Conclusions

Microbial diversity in anterior intestine was higher than in posterior intestine.

Proteobacteria and *Actinobacteria* were the most predominant phyla in anterior intestine, whereas *Spirocchaetes* and *Brevinema* were the most frequent genus detected in posterior intestine.

FEMS7-1415
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DETECTION, ISOLATION AND CHARACTERIZATION OF PLASMID DNA FROM FISH PATHOGENIC AND PROBIOTIC SHEWANELLA PUTREFACIENS STRAINS

M. Maqueda Dominguez[2], S.T. Tapia-paniagua[1], E. Alcaide[3], C. Esteve[3], M.D.C. Balebona[4], M.Á. Moriñigo[4]
[1]Universidad De Malaga, Microbiologia, Malaga, Spain
[2]Universidad De Malaga, Microbiology, Malaga, Spain
[3]University Of Valencia, Departament Of Microbiology And Ecology, Valencia, Spain
[4]University Of Malaga, Microbiology, Malaga, Spain

Backgrounds

Plasmids are important vehicles for rapid adaptation of bacteria to changing environments, providing beneficial accessory traits, such as degradative pathways and pathogenicity determinants. *Shewanella putrefaciens* Pdp11 (SpPdp11) is proposed as probiotic for farmed fish and for this reason, the evaluation of the plasmid content in this bacteria is important for its future applications in aquaculture.

Objectives

The objective of this study was the detection of plasmids in SpPdp11 cells to carry out the comparison to those detected in *S. putrefaciens* pathogenic strains.

Methods

SpPdp11 was isolated from healthy gilthead seabream, whereas pathogenic *S. putrefaciens* strains (SH2, SH4, SH6, SH16 and SH19) were isolated from diseased eels. For isolation of plasmid DNA from *S. putrefaciens* the strains were grown in trypticase soy broth, supplemented with 15% sodium chloride. Plasmids DNA from bacterial cells were isolated using Plasmid DNA miniprep kit (Thermo Scientific, Germany). An agarose gel electrophoresis (1% agarose, 0.5% TAE) was performed for the separation of the plasmid DNA at 50 V for 1 h. The molecular weight was calculated using the equivalence 1.0 µM = 2.07 x 10^6 Daltons. Restriction enzymes Sau3AI and SauII were used to digest the plasmids with the same molecular weight and the fragments were separated by agarose gel electrophoresis.

Conclusions

Two different plasmids were detected in SpPdp11 strain whereas only two pathogenic strains contained plasmids, with a different one each of them.

None of these plasmids corresponded to those detected in SpPdp11.

FEMS7-2774
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

COMPOSITION OF THE MICROBIOTA PRESENT IN SKIN MUCUS FROM ULCERATED AREAS OF SPARUS AURATA AND COMPARISON WITH HEALTHY SKIN.

S.T. Tapia-Paniagua[1], M. Fumanal[1], H. Cordero[2], M.C. Balebona[3], X. Moreno Ventas[3], M.Á. Esteban[4], M.Á. Moriñigo[5], D. Ceballos-Francisco[4]
[1]Universidad De Malaga, Microbiologia, Malaga, Spain
[2]Faculty Of Biology- University Of Murcia-, Department Of Cellular Biology And Histology., Murcia, Spain
[3]University Of Malaga, Microbiology, Malaga, Spain
[4]University Of Murcia, Department Of Cell Biology And Histology, Murcia, Spain
[5]University Of Malaga, Microbiology, Malaga, Spain

Backgrounds

Skin is the first line of defense, but alike to mammals, fish skin can be considered a mucosal tissue. Teleost fish skin has shown to contain associated lymphoid tissue similar to that of the gut-associated lymphoid tissue, and including antimicrobial peptides, lectins, assorted pathogen recognition receptors (PRR), lysozymes, immune respondents and similarly to the fish gut it harbors a microbiota. The interest in understanding components of the mucosal immune system of fish is growing but it is still limited.

Objectives

The objective of this study has been to evaluate the composition of the microbiota detected in ulcers of skin of gilthead seabream (*Sparus aurata*) and to compare it with the skin microbiota of healthy seabream.

Methods

Skin were collected from experimental chronic ulcers and non-injury areas of healthy specimens of gilthead seabream (*Sparus aurata* L.). DNA was extracted from samples and was sent to ChunLab (Seoul, Korea) to determine the microbial DNA sequences of the 16S rRNA gene by Illumina technology. Sequences were analyzed using CLcommunity™ software (ChunLab). The relative abundance of microbial clades at different taxonomic levels was calculated and used to perform the comparative distribution analysis.

Conclusions

Significant differences were detected in the microbiota from ulcerated skin in comparison with health skin: (1) increases of γ–Proteobacteria such as Vibrionales and Alteromonadales; (2) levels of members of Bacteroidetes and Firmicutes such as Bacteroidaceae and Lactobacillaceae decreased, while Prevotallaceae and Streptococcaceae were increased.

FEMS7-0297
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

RHEOLOGICAL CHARACTERIZATION OF IN SITU YEAST BIOFILMS

M.C. Tarifa[1], *L.I. Brugnoni*[2], *J.E. Lozano*[1], *D. Genovese*[1]
[1]*Planta Piloto de Ingeniería Química CONICET-UNS, Ingeniería de Alimentos, Bahía Blanca, Argentina*
[2]*Instituto de Ciencias Biológicas y Biomédicas del Sur CONICET-UNS, Microbiología Industrial y de los Alimentos, Bahía Blanca, Argentina*

Backgrounds

In industrial processes biofilms are grown under a wide distribution of local hydrodynamic strengths; consequently biofilms are characterized by their thickness, architecture, and rheological properties.

Objectives

To study the response in the mechanical properties of yeast biofilms to variations in flow conditions and food matrix.

Methods

A suspension of *Candida tropicalis, C.krusei, C.kefyr* and *Rhodotorula mucilaginosa* isolated from juice ultrafiltration membranes, was done in apple and pear juice (≈10^6 cells/ml of each strain). Biofilms were done on stainless steel surfaces for a period of one month under static and turbulent flow, and two juice concentrations (6 and 12 °Brix). Samples were taken off the system for rheological measurements, counts and SEM processing. Viscoelastic properties were determined by small deformation dynamic oscillatory measurements in a Paar Physica rheometer MCR301, using parallel plates, whereas CHROM agar *Candida* was used for counting.

Conclusions

The results showed that yeast biofilms had a predominately solid-like behaviour indicating gel-type structures, being G' higher than G" at all frequencies studied. Average loss tangent (tan δ) values were low (< 0.25) and approximately independent of frequency. Under turbulent flow biofilms were structurally stronger than under static conditions and presented higher counts (at least 0.5 Log units). The only specie not recovered was *R. mucilaginosa*; the biofilms formed presented tighter structures when subjected to a flow. Rheology determines the mechanical stability of biofilms, its knowledge is crucial to fully interpret their behavior. From our results the type of flow was de parameter that most influenced the development and strength of the biofilms.

FEMS7-0483
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IMPACT OF FLOW CONDITIONS AND SURFACE TYPE ON THE RESISTANCE OF CANDIDA TROPICALIS BIOFILMS TO DISINFECTION

M.C. Tarifa[1], *B. Pitts*[2], *P. Stewart*[2]
[1]*Planta Piloto de Ingeniería Química CONICET-UNS, Ingeniería de Alimentos, Bahía Blanca, Argentina*
[2]*Center for Biofilm Engineering, Chemical & Biological Engineering, Bozeman, USA*

Backgrounds

While biofilm formation is a general characteristic of microbes, features such as architecture, matrix composition, and resistance to antimicrobials are species and substrate dependent.

Objectives

The aim was to delve into the response in thickness, architecture, and mechanical resistance of *Candida tropicalis* biofilms.

Methods

The strain used was *Candida tropicalis* ATCC 13803, which was cultivated in SDB for 48 and 72 h on stainless steel (SS) and polycarbonate coupons (4.05 cm^2). Two flow conditions were tested: turbulent flow with a CDC reactor (Biosurface Technologies, USA) (flow rate of 4 ml/min, speed of 200 rpm); and for static flow 12 wells plates were used. Coupons were subjected to the analysis of: mechanical resistance in a flow cell, counting, CLSM imaging and cryosectioning. For disinfection, coupons were treated with solutions of 200 ppm of NaClO for 15 minutes, and then neutralized in sodium thiosulfate 0.2%.

Conclusions

Biofilms were observed on SS and polycarbonate at 48 and 72 h after cell staining with LIVE/DEAD® Kit, increasing with Re and time. Flow conditions impacted biofilm architecture and thickness, under turbulent flow biofilms presented mushroom shapes with hyphae and the largest increase in thickness (> 90 µm), whereas static flow presented homogeneous layers of rounded cells (up to 30 µm). The results underline that the biofilm resistance to NaClO depended on the growth time, the surface type and the type of flow. At 48 h log reductions ranged between 0.2-0.58 and 1.2-1.36 for turbulent and static flow, respectively whereas at 72 h were of 0.32-1.7 and 2.65-2.82.

FEMS7-2394
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

GUT MICROBIOTA DINAMICS IN CARNIVOUROUS FISH FED PLANT-BASED DIETS

C. Serra[1], P. Enes[1], A. Oliva-Teles[1,2], F. Tavares[2,3]
[1]CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal
[2]FCUP – Faculdade de Ciências- Universidade do Porto, Departamento de Biologia, Porto, Portugal
[3]CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO- Laboratório Associado- Universidade do Porto, Vairão, Portugal

Backgrounds

A major challenge in carnivorous fish aquaculture production is the substitution of fish meal (FM), an unsustainable commodity and a source of organic pollutants, by plant feedstuffs (PF). PF have a limited nutritive value due to the presence of non-starch polysaccharides (NSP) which are not metabolized by fish. Thus, a selective pressure of NSP-enriched diets towards gut microbes with NSP-hydrolytic potential is expected to occur, helping the fish host to obtain energy from otherwise indigestible dietary constituents.

Objectives

Comprehensively assess the microbiota dynamics of carnivorous fish species in response to the dietary incorporation of plant feedstuffs.

Methods

Triplicate groups of European sea bass juveniles were fed a FM-based diet (control) or 3 PF-based diets (SBM, soybean meal; RSM, rapeseed meal; SFM, sunflower meal) during 6 weeks, before recovering intestinal samples for microbiota composition analysis using the Illumina´s MiSeq platform. QUIME software was used for taxonomy assignment and diversity analysis, and one-way ANOVA statistical analysis was carried out with IBM SPSS Statistics 24 and STAMP v2.1.3 software.

Conclusions

A significant (p=0.020) decrease in the *Chao1* species richness estimator index was observed in the gut samples of SBM and RSM experimental fish. The reduction on microbiota richness was accompanied by a decline on the relative abundance of *Acidobacteria* (p=0.030), *Elusimicrobia* (p=0.028) and *Nitrospirae* (p=0.010) phyla. On contrary, SBM and RSM PF-based diets seem to favor the *Firmicutes* (p=0.01), in particular the Bacillaceae (p=0.017) and Clostridiaceae (p=0.007), two bacterial families known to harbor carbohydrate active enzymes and thus putatively more prone to grow in high NSP environments.

FEMS7-2000
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

STRAIN-LEVEL MICROBIAL POPULATION STRUCTURES FROM SHOTGUN METAGENOMICS USING STRAINPHLAN

A. Tett[1], D.T. Truong[1], E. Pasolli[1], C. Huttenhower[2], N. Segata[1]
[1]University of Trento, CIBIO, Trento, Italy
[2]Harvard School of Public Health, Biostatistics Department, Boston, USA

Backgrounds

It is becoming increasingly evident that crucial microbiome factors associated with human health and disease occurs at the level of microbial strains. Metagenomics offers the opportunity to characterise the communities at a strain-level resolution but appropriate computational methods are required.

Objectives

To fully exploit the potential of metagenomic sequencing we developed StrainPhlAn, a novel metagenomics strain identification approach, based on per-sample dominant sequence variant reconstruction within species-specific marker genes. It provides a strain-level phylogenetic overview of the microbial diversity within and between metagenomic samples.

Methods

We applied StrainPhlAn on lung metagenomes of cystic fibrosis patients and thousands of publically available gut metagenomes. Across metagenomes StrainPhlAn identified subject-specific strain variants (<5% inter-subject strain sharing), that are remarkably stable, with the dominant strain for each species being retained over time (for >70% of species). Applied to gut metagenomes, we found strong evidence of biogeographically correlated population structures suggesting intriguing hypotheses about their population biology. For instance, two common but poorly characterised gut species, *Eubacterium rectale* and *Prevotella copri,* display a discrete subspecies population structure compared to others such as *Faecalibacterium prausnitzii* which has a more continuous genetic variation across and within human populations. We further estimated the genetic variability of different gut inhabitants, with *Bacteroides* species showing relatively low species divergence compared to *P. corpi*, one of the most plastic gut colonisers.

Conclusions

StrainPhlAn has enabled the population structures of previously poorly characterised or inaccessible microbes to be studied providing a comprehensive strain level overview of lung and gut associated microbes.

FEMS7-2119
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DETERMINIG THE INFLUENCE OF SOIL PH ON THE ABUNDANCE, DIVERSITY AND ACTIVITY OF COMAMMOX AND CLASSICAL NITRITE-OXIDISING BACTERIA

C. Thion[1], Y. Meng[2], P. Pjevac[3], J.I. Prosser[2], H. Daims[3], G.W. Nicol[1]
[1]Ecole Centrale de Lyon, Laboratoire Ampère, ECULLY cedex, France
[2]Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, United Kingdom
[3]Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Vienna, Austria

Backgrounds

For over 100 years, aerobic nitrification was thought to be a separated two-step process, with ammonia oxidised to nitrite by ammonia oxidisers (AO), which was subsequently oxidised to nitrate by nitrite oxidising bacteria (NOB), including members of the *Nitrospira* and *Nitrobacter* genera in soil. This dogma was recently challenged by the discovery of *Nitrospira* performing both steps, or 'complete ammonia oxidation' (comammox), with representatives placed within two clades (A and B). Soil pH is a major factor controlling the distribution/activity of AO, however the role of pH influencing the distribution of NOB and comammox *Nitrospira* is unclear.

Objectives

Our objective was to determine how soil pH influences the distribution and activity of soil NOB, testing the hypothesis that *Nitrobacter* and *Nitrospira* (including comammox) communities in soils have contrasting pH preferences.

Methods

Using a long-term soil pH gradient, the community structure, abundance and activity of NOB were determined. qPCR and high-throughput sequencing of functional genes (nxrA, *nxrB*, *amoA*) demonstrated both *Nitrospira* and *Nitrobacter* were found in all soils, with distinct, pH-adapted populations within each genus. Only commamox clade B group was prevalent in soil, with their distribution congruent with that of all *Nitrospira*, i.e. low abundance (or not detected) in acidic soils and more abundant at neutral pH.

Conclusions

Nitrospira populations increased in both diversity and abundance with increasing soil pH, indicating that comammox *Nitrospira* are not likely to be major contributors to nitrification in low pH soils, but may make a major contribution to this process in neutral or alkaline soils.

FEMS7-2640
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EVOLUTION AND FUNCTION OF EUKARYOTIC-LIKE PROTEINS IN MICROBIAL SPONGE SYMBIONTS

T. Thomas[1], M. Nguyen[1], D. Reynolds[1], C. Diez-Vives[1], L. Moitinho e Silva[1], S. Nielsen[1], R. Thangamani[1]
[1]University of New South Wales, Centre for Marine Bio-Innovation, Sydney, Australia

Backgrounds

Sponges form ancient and complex symbiotic interactions with microorganisms. How these interactions are mediated on a molecular level is however poorly understood. Our recent (meta-) genomic analyses have uncovered the surprising presence and abundance of genes encoding for eukaryotic-like proteins (ELPs) in microbial symbionts of sponges.

Objectives

Here we hypothesise that these ELPs have an important function in mediating molecular interactions between sponges and their microbial symbionts.

Methods

We applied a range of phylogenetic, –omics and molecular techniques to describe the evolution and function of ELPs.

Conclusions

Phylogenetic analyses indicate that these ELPs have been horizontally transferred from sponges and other eukaryotes into symbiont lineages. Meta-transciptomic analyses showed that a large array of different ELPs is expressed under natural settings, indicating their functional role in symbiosis. Through recombinant approaches it was shown that ELPs can inhibit eukaryotic phagocytosis, which is a relevant phenotype for a symbiont to avoid being consumed by sponge amoebocytes. In fact, *Escherichia coli* cells, which recombinantly express ELPs from sponge symbionts, were able to persist inside sponges, while *E. coli* without ELPs were rapidly consumed. For some symbiont ELPs it was also shown that they specifically bind to sponge proteins involved bacterial recognition and degradation.

Overall these results support a model whereby microbial symbionts have acquired eukaryotic host genes that have subsequently evolved to function as molecular mediators of symbiotic interactions.

FEMS7-0396
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ANAEROBIC OXIDATION OF METHANE ASSOCIATED WITH SULFATE REDUCTION IN A NATURAL FRESHWATER GAS SOURCE

P. Timmers[1], D. Suarez-Zuluaga[2], M. van Rossem[1], M. Diender[1], A. Stams[1], C. Plugge[1]
[1]Wageningen University, Laboratory of Microbiology, Wageningen, The Netherlands
[2]Wageningen University, Environmental Technology, Wageningen, The Netherlands

Backgrounds

Anaerobic oxidation of methane (AOM) coupled to sulfate reduction (SR) occurs in marine sediments and is catalysed by anaerobic methane oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) of the SEEP-SRB1 clade of the Deltaproteobacteria. AOM can also be coupled to other electron acceptors, such as nitrate and metal-oxides (ferrihydrite, birnessite).

Objectives

In freshwater, AOM coupled to SR is thought to be limited by low sulfate concentrations, and has therefore not been extensively studied. Moreover, it is not known if different organisms are involved in freshwater AOM compared to marine environments. We therefore investigated the occurrence of AOM coupled to SR and the organisms involved in a freshwater natural gas source.

Methods

Freshwater samples were used in long-term incubations with and without $^{13}CH_4$ and with and without added electron acceptors (nitrate, sulfate, ferrihydrite, humic acids). After 323 days, archaeal and bacterial 16S rRNA gene profiling and qPCR analysis with specific primers for ANME archaea were performed.

Conclusions

Only with sulfate as electron acceptor, net AOM occurred and sulfide increased simultaneously with $^{13}CO_2$ production. Archaeal 16S rRNA gene analysis showed more ANME-2a/b sequences in incubations with methane and sulfate as compared with only methane addition. Higher abundance of ANME-2a/b in incubations with methane and sulfate as compared with only sulfate addition was shown by qPCR analysis. Bacterial 16S rRNA gene analysis showed presence of SRB belonging to SEEP-SRB1. This is the first report of an enrichment culture of ANME-2a/b and SEEP-SRB1 from a freshwater environment where AOM is associated with SR.

FEMS7-1952
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BACTERIAL COMMUNITY SHOWS HIGHLY ADAPTED GROUPS AND VARIOUS LIFE STRATEGIES DURING THE PROCESS OF DEADWOOD DECOMPOSITION

V. Tláskal[1], L. Štercová[1], T. Vrška[2], P. Baldrian[1]
[1]Institute of Microbiology - Academy of Sciences of the Czech Republic, Laboratory of Environmental Microbiology, Prague, Czech Republic
[2]The Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Department of Forest Ecology, Brno, Czech Republic

Backgrounds

Nutrients fixed by photosynthesis in tree biomass are in significant amounts released after death of a tree in the process of deadwood decomposition. This process is driven by fungi and bacteria - microorganisms with several tools for degradation of recalcitrant plant polymers and compounds of microbial origin. However, the contribution of bacteria is far less understood than that of fungi.

Objectives

The aim of this work was to explore bacterial communities in logs of Abies alba and Fagus sylvatica from different phases of decomposition in the natural temperate forest in the Czech Republic and to identify drivers of community assembly.

Methods

Amplicon sequencing of 16S rRNA was used for characterization of community composition while deadwood censuses were the source of log characteristics and their history. Bacterial biomass and fungal/bacterial ratios were followed as well as wood chemistry.

Conclusions

Bacterial taxa typical for fresh deadwood showed the ability to decompose cellulose (*Cellulomonas*) and to fix nitrogen (*Methylocapsa*). Strategies for utilization of fungal biomass as a nutrient source (*Chitinophaga*) and association with mycosphere and rhizosphere (*Steroidobacter*) occurred in taxa present in older deadwood. Co-occurrence pattern analysis showed links between the occurrence of bacterial and fungal taxa either due to mycophagy or due to specialization for degradation of fungal metabolites by bacteria. Bacterial community appears to be driven more by fungal community and deadwood age rather than by tree species or spatial proximity of logs. Nitrogen fixation and mycophagy may represent bacterial strategies to overcome nitrogen limitation. Both strategies may have significant impact on ecosystem functioning.

FEMS7-1451
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIOREMEDIATION POTENTIAL OF ENDOPHYTIC FUNGI MUCOR SP. ISOLATED FROM EICHHORNIA CRASSIPES (MART.) (PONTEDERIACEAE)

T. Tognolli de Almeida[1], J.L. de Azevedo[1], M.C. Quecine[2]
[1]University of São Paulo, Center of Nuclear Energy in Agriculture CENA/USP, Piracicaba, Brazil
[2]University of São Paulo, Department of Genetics- Luiz de Queiroz College of Agriculture, Piracicaba, Brazil

Backgrounds

Endophytic fungi live in the interior of plant tissues or organs, without causing harm to their hosts. The several interesting characteristics of these microorganisms include their bioremediation potential.

Objectives

This study aimed to examine the bioremediation potential of endophytic fungi *Mucor* sp. isolated from *Eichhornia crassipes*, through the absorption ability and fungal tolerance to the heavy metal cadmium.

Methods

For the evaluation of tolerance to the heavy metal cadmium, 6-mm plugs of endophyte were inoculated onto Potato dextrose Agar (PDA) Petri dishes supplemented with 250, 500, 1000 and 2000 mg/L of heavy metal Cadmium sulfate, incubated under B.O.D. at 28 ºC for 7 days. The metal tolerance index (Ti) was estimated by measuring the radial colony extension. For evaluating absorption ability of cadmium, Potato dextrose broth (PDB) containing 200, 400, 600, 800 and 1000 mg/L of cadmium sulfate was dispensed in 100 mL in conical flasks and inoculated with 6-mm plugs of endophyte and incubated at 28 ºC for 3, 9 and 15 days. The fungal growth was harvested through filtration. Then dried at 80 ºC for 18 h, the heavy metal concentration was estimated by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Conclusions

The metal tolerance index was highest (Ti >0.4) at 1000 mg/L. Regarding an increased cadmium biosorption, the highest absorption was 956 mg/g after 15 days of incubation.Therefore the results indicated that endophytic isolate *Mucor* sp. demonstrated the ability to retain the cadmium indicating its potential to be utilized for remediation of cadmium.

FEMS7-0883
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

A NOVEL QUORUM-QUENCHING ENZYME IDENTIFIED IN A HYPERSALINE SOIL

M. Torres[1,2], S. Uroz[3], R. Salto[4], L. Fauchery[3], I. Llamas[1,2]
[1]University of Granada, Microbiology, Granada, Spain
[2]Institute of Biotechnology, Biomedical Research Center, Granada, Spain
[3]French National Institute for Agricultural Research INRA, Interactions Arbres-Microorganismes, Nancy, France
[4]University of Granada, Biochemistry and Molecular Biology, Granada, Spain

Backgrounds

Quorum sensing (QS) is an intercellular communication system mediated by signal molecules which is used by many bacteria to coordinate their behaviour. In order to interfere with QS, many of the competing organisms have developed different strategies, such as the enzymatic inactivation of the signal molecules, known as quorum quenching (QQ).

In recent years, the QS and QQ mechanisms have been described in numerous bacterial species. Nevertheless, there is very little data concerning these systems of regulation in hypersaline environments.

Objectives

The aim of this study is the identification and characterization of new QQ enzymes in a hypersaline soil of Spain by using a cultivation-independent approach.

Methods

In this communication we present the construction of a metagenomic library of 250,000 clones and the screening of its QQ activity. One clone was selected that allowed the degradation of a wide range of AHL-type signal molecules. The sequencing of the fosmid in the positive clone revealed a 42,318 bp environmental insert characterized by 46 ORFs. The sub-cloning of these ORFs demonstrated that a single gene (*hqiA*) allowed AHL degradation. Bio-informatic analyses highlighted that HqiA showed no sequence homology with the known prototypic AHL enzymes, expanding the AHL-lactonase family.

Conclusions

This study represents the first fosmid-based screening of a hypersaline soil metagenome to search for genes involved in QQ. A novel type of AHL-degrading enzyme has been identified, not previously related to the known prototypes. It has been tested *in vivo* and it is a very promising tool with biotechnological applications.

FEMS7-0889
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

GENOME ANALYSIS OF TWO ALTEROMONAS STELLIPOLARIS STRAINS WITH QUORUM QUENCHING ACTIVITY

M. Torres Bejar[1,2], T.M. Chong[3], K.W. Hong[3], K.G. Chan[3], Y. Dessaux[4], I. Llamas[1,2]
[1]University of Granada, Microbiology, Granada, Spain
[2]Institute of Biotechnology, Center for Biomedical Research, Granada, Spain
[3]University of Kuala Lumpur, Medicine, Kuala Lumpur, Malaysia
[4]National Center of Scientific Research CNRS, Microbiology, Gif-sur-Yvette- Paris, France

Backgrounds

The massive use of antibiotics has resulted in the development of resistant bacterial strains and ineffective antibiotic treatments. Therefore, new strategies to control bacterial infections are urgently needed and sought after. Nowadays, research is focused on finding alternatives to fight bacterial infections. One of them relies upon enzymatic degradation (quorum quenching, QQ) to target the intercellular communication systems (quorum sensing, QS) that regulate the production of virulence factors in pathogenic bacteria.

Since marine environments have been demonstrated to be an enormously rich source of new secondary metabolites to fight diseases, a bivalve hatchery in Granada (Spain) was analyzed, which allowed the selection of two *Alteromonas stellipolaris* strains showing QQ activity *in vivo*.

Objectives

The aim of this study is the sequencing and analysis of the genomes of two marine *A. stellipolaris* strains that have been reported to present QQ activity.

Methods

The genomes of the two marine strains were sequenced by single molecule real-time sequencing approach. In both genomes, genes encoding a QQ enzyme (acylase PvdQ) were identified. Additionally, in one of the strains a non-ribosomal peptide synthase (Nrps) cluster with very low homology compared to previously known Nrps clusters was identified. This cluster could be involved in the synthesis of a natural product of biotechnological interest.

Conclusions

The genome analysis of the two QQ strains has confirmed the presence of an acylase that degrades signal molecules and has provided information related with the production of compounds with a wide perspective of applications.

FEMS7-0298
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

FUNCTIONAL MICROBIAL FEATURES DRIVING COMMUNITY ASSEMBLY DURING SEED GERMINATION AND EMERGENCE

G. Torres-Cortés[1], S. Bonneau[1], O. Bouchez[2], C. Genthon[2], M. Briand[1], M.A. Jacques[1], M. Barret[1]
[1]Institut de Recherche en Horticulture et Semences INRA, Equipe EmerSys, BEAUCOUZE cedex, France
[2]GeT-PlaGe INRA, Genotoul, Castanet-Tolosan, France

Backgrounds

Plant-associated microorganisms have a decisive role in host adaptation and affect plant productivity. Due to this important role, numerous studies have been focusing on the microbes associated with the rhizosphere or phyllosphere. However, little is known about the role of microbes associated with other plant habitats such as the seed. Microbial interactions occurring on and around the seed are especially important for plant fitness since seed-borne microorganisms are the primary source of inoculum for the plant.

Objectives

The aim of this study is to uncover functional microbial traits implicated in the assembly of the seed microbiota during seed germination and emergence.

Methods

With this purpose, we performed shotgun sequencing of microbial DNA from seeds, germinating seeds and seedlings.

Conclusions

Metagenomic read composition-based classification showed significant changes in microbial community during emergence. Moreover, by predicting the functional profile of these communities, we observed a link between taxonomic changes and functional traits that could lead into niche-specific adaptations. In particular, we found a cluster of microbial functions enriched at emergence (secretion systems, secondary metabolism…) that may be important for microbial colonization and subsequent resilience on seedlings. In addition, we used the generated data for the reconstruction of population genomes drafts of the most significant seed-colonizer taxa. Altogether, these data can be used as molecular markers to select microbial inoculants possessing full genetic potential to colonize and persist in these plant habitats.

FEMS7-0469
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MATERNAL INFLUENCE ON THE GUT MICROBIOME EXCEEDS GENETIC CONTRIBUTION DURING EARLY MOUSE DEVELOPMENT

N.S. Treichel[1], A. Schöler[1], Z. Prevoršek[2], B. Stres[2], M. Schloter[1]
[1]Helmholtz Zentrum München GmbH, Comparative Microbiome Analyses, Muenchen, Germany
[2]University of Ljubljana, Department of Animal Science, Ljubljana, Slovenia

Backgrounds

The microbiota of the gut is strongly linked to the phenotype of the host. It is acquired after birth and involves both maternal and environmental contributions. Also the genetic structure of the host was shown to influence the gut microbiome.

Objectives

This study aimed to investigate to what extent the genetics of an individual and the microbiota inherited from the mother have an impact on the gut microbiome composition of the offspring.

Methods

Therefore a cross-fostering experiment involving two genetically different mouse lines was conducted. The mice were sacrificed at three weeks of age and their colonic microbiome was analyzed by paired-end next generation sequencing. The sequences were taxonomically affiliated and analyzed at 97% sequence identity with QIIME using the Greengenes database.

Conclusions

The abundance of several bacterial families was influenced by the genotype of the host or by the kind of inherited microbiome. Beta-diversity analysis showed a higher impact of the maternal microbiome on the gut microbiome composition, than the host's genetic background. The analysis of co-presence networks further revealed a contribution of the genotype to the OTU structure of the networks, while the inherited microbiome determined the connectivity of the network.

This study reveals that at an early stage of development the bacteria derived from the mother have a higher impact on the gut microbiome composition of an individual, than the genetic structure of the individual.

FEMS7-2377
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INTER-ANNUAL PREDICTABILITY OF ATMOSPHERIC MICROORGANISMS DYNAMICS AND OCCURRENCE OF AIRBORNE POTENTIAL PATHOGENS

X. Triadó-Margarit[1], J. Caliz[1], L. Camarero[1], E.O. Casamayor[1]
[1]Center for Advanced Studies of Blanes CSIC, Integrative Freshwater Ecology Group, Blanes, Spain

Backgrounds

Microbes represent an important component of atmospheric aerosols with the potential to impact human health. However, our current knowledge on the temporal dynamics of airborne microbial populations remains poorly explored, and no studies have consistently addressed these questions under the long-term.

Objectives

We aimed to (i) describe in detail changes of the airborne microbial diversity and community composition over time, (ii) determine whether or not temporal variability in atmospheric microbial communities is predictable, (iii) estimate the temporal dynamics of the potentially pathogenic airborne microbes.

Methods

We applied high-throughput sequencing techniques (16S rRNA and 18S rRNA genes amplicons) to characterize microorganisms from wet depositions collected at a high elevation site (Central Pyrenees, NE Spain) over a period of seven years. We assessed the presence of potentially pathogenic microbes after comparison against in-house databases of previously reported obligate and opportunistic pathogens. The origin of aerosols was estimated by chemical analyses of rain and snow.

Conclusions

Airborne microbial communities from the same season were more similar each other along the inter-annual study period. Low overlapping was observed between summer and winter communities. Indicative taxa, including a set of potentially pathogenic microbes, were identified for these two seasons. Phytopathogenic fungi and opportunistic bacteria were the most abundant potential pathogens. Overall, the long-term analysis unveiled regularities on the microbial community structure over time and seasonal recurrence of specific airborne microbes.

FEMS7-2583
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

VARIATIONS IN ECOLOGICAL PROCESSES INFLUENCING THE ASSEMBLY OF BACTERIAL COMMUNITIES IN SUCCESSIONAL ENVIRONMENTS

B. Tripathi[1], J. Stegen[2], M. Kim[1], D. Ke[3], J. Adams[3], Y.K. Lee[1]
[1]Korea Polar Research Institute, Polar Life Sciences, Incheon, Republic of Korea
[2]Pacific Northwest National Laboratory, Biological Sciences, Washington, USA
[3]Seoul National University, Biological Sciences, Seoul, Republic of Korea

Backgrounds

Understanding the fundamental ecological processes that shape the assembly of microbial communities is a major challenge in microbial ecology. Successional soil environments offer a model setting for understanding the ecological processes governing the assembly of microbial communities

Objectives

As pH often changes during succession, this could be the main cause of the observed trends in bacterial community assembly and also influence the relative importance of stochastic and deterministic processes during succession. We tested this hypothesis by carrying out a meta-analysis of a range of different ecological successional environments, not all of which show the same trend in soil pH over time.

Methods

We applied an ecological null modeling approach to analyze the phylogenetic bacterial community assembly and processes.

Conclusions

We found that soil pH was the best predictor of bacterial community assembly and the relative importance of stochastic and deterministic processes along successional environmental gradients. This was irrespective of successional stage (early or late) and time scale (short or long-term). Extreme acidic or alkaline pH conditions lead to assembly of a more phylogenetically clustered bacterial community through deterministic processes, whereas pH conditions close to neutral lead to a less phylogenetically clustered bacterial community with more stochasticity. We suggest that the influence of pH, rather than successional age, is the main driving force in producing trends in phylogenetic assembly of bacteria, and that this also influences the relative balance of stochastic and deterministic processes along successional gradients.

FEMS7-1769
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITY OF PLANT EXTRACTS AGAINST PATHOGENS CAUSING SKIN INFECTIONS

M. Mishra[1], S. Sharma[1], D. Tripathi[1]
[1]Motilal Nehru National Institute of Technology Allahabad,
Centre for Medical Diagnostic and Research & Department of Biotechnology, Allahabad, India

Backgrounds

Availability of plant derived compounds having medicinal importance is gaining much importance since past few decades. In lieu of importance of plant extract's role in curing skin infections, present work is focused on antimicrobial and anti-inflammatory efficacy of extracts of *Citrica medica, Datura metel, Juniperus communis* against panel of microbes implicated in skin diseases.

Objectives

To evaluate antimicrobial and anti-inflammatory activity of plant extracts for the treatment of skin infections.

Methods

Different plant parts of *Citrica medica, Datura metel, Juniperus communis* in solvents chloroform, ethanol, methanol and water are used for extraction purpose through soxhlet method and obtained extract was filtered and evaporated in hot air oven. The phytochemical analysis for active constituents of these extracts was implicated through thin layer chromatography. MICs, MBC and MFC were determined for antimicrobial and antifungal activities of extract against *P. aeruginosa, S. aureus* and *C. albicans* respectively. Anti-inflammatory activity was determined by Bovine serum albumin denaturation technique.

Conclusions

The extract of these herbal plants has showed potent anti-inflammatory & antimicrobial activity against *P. aeruginosa, S. aureus* and *C. albicans. In silico* analysis for the interactions of phytoconstituents with microbial proteins and their molecular dynamics simulation, prediction of biological activities will be accomplished using computational studies. After the computational studies the extracts will be analyzed for their effectiveness against skin infections followed by development of bioformulations that can be used commercially for skin disease treatment. The effectiveness of the bioformulations after their characterization and toxicity analysis will be accomplished both *in vitro* and *in vivo.*

FEMS7-1743
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INVESTIGATION OF THE BIOLOGICAL ACTIVITY INDICATORS FOR MILITARY CONTAMINATED SOIL

A. Nurzhanova[1], V. Pidlisnyuk[2], K. Abit[3], R. Aligulova[1], J. Trögl[2]
[1]Institute of Plant Biology and Biotechnology, IPBB, Almaty, Kazakhstan
[2]Jan Evangelista Purkyně University in Ústí nad Labem - Faculty of the Environmen, Department of Technical Sciences, Ústí nad Labem, Czech Republic
[3]Al-Farabi Kazakh National University, afknu, Almaty, Kazakhstan

Backgrounds

Military pollution affects soil microbial communities.

Objectives

Evaluate the level of pollution on the biological activity of military contaminated soil in Kazakhstan (Maili village, Almaty region).

Methods

Determination of heavy metals in the research soils was conducted at inductively coupled plasma mass spectrometry ICP-MS Agilent 7500. Investigation of soil biological motions: urease and cellulolytic activities was done by Aristovkaya express method.

Conclusions

Research soil was polluted by heavy metals: concentrations of As exceed maximum permission level in 5 times, Cu – 13 times, Sr – 22 times, Pb – 29 times, Zn – 11 times. Index of total geochemical contamination varied from 37.1 to 71.7 mg/kg and indicated hazardous state of the research soil. Heavy metals determined in the soil had a depressing effect to the reaction of urease and cellulolytic activities. Cellulolytic activity was reduced to 14% compared to 30% for the control soil. The weak increasing of urease activity determined by pH changing within 24 hours was observed for the military soil (pH 9) in comparison with control one (pH 12).
Soil at the military site is dangerous for the environment and human. Heavy metals inhibited the biochemical activity and reduced fertility and self-purification of the soil.

FEMS7-1108
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

NEGATIVE CHEMOTAXIS RESPONSE TOWARD MALEATE IN BACTERIAL WILT PATHOGEN, RALSTONIA SOLANACEARUM

M. Tunchai[1], *A. Hida*[1], *T. Tajima*[1], *J. Kato*[1]
[1]*Hiroshima University, Molecular Biotechnology, Higashi-Hiroshima, Japan*

Backgrounds

Ralstonia solonacearum, the causal agent of bacterial wilt disease, is regarded as one of the world's most destructive plant pathogenic bacteria.

Objectives

Previous studies found that chemotaxis is an essential trait for the early stage of host invasion and pathogenic fitness in *R. solanacearum*, so we have attempted to functionally characterize chemoreceptors, so called methyl-accepting chemotaxis proteins (MCPs), in this pathogen.

Methods

R. solanacearum strain Ps29 exhibited strong negative chemotactic response to a nonmetabolizable maleate. Like most of reported repellents, maleate is harmful to *R. solanacearum* by reducing its growth rate. Result of quantitative chemotaxis assay of *R. solanacearum* Ps29 with various concentration of maleate showed that repellant strength increased as concentration increasing. To identify MCP potentially sensing maleate, single *mcp* gene-deletion mutant library of strain Ps29 was screened. Among 22 mutants, only Δ*mcp16* mutant strain failed to exhibit chemotactic response to maleate, whereas the other mutants showed no significant difference in responses comparing with that of wild-type strain. The Δ*mcp16* mutant harboring the *mcp16* gene on plasmid exhibited wild-type chemotaxis response toward maleate.

Conclusions

These results indicated that *mcp16* encodes chemotactic transducer mediating negative response to maleate. Protein Blast analysis using the putative ligand binding domain (LBD) of Mcp16 as a query sequence revealed that *R. solanacearum* species have Mcp16 orthologs of which LBDs are highly similar to that of strain Ps29 (more than 75% identity). This work contributes to expand the range of characterized chemotactic transducers and their ligands in bacterial wilt pathogen *R. solanacearum*.

FEMS7-0482
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE ECOLOGICAL ROLE OF VOLATILE AND SOLUBLE SECONDARY METABOLITES PRODUCED BY SOIL BACTERIA

O. Tyc[1], *P. Garbeva*[1]
[1]*Netherlands Institute of Ecology NIOO-KNAW, Microbial Ecology, Wageningen, The Netherlands*

Backgrounds

In soil ecosystems, bacteria live in proximity with many different species and form complex, species rich communities. Within those communities, bacteria produce and release a vast number of secondary metabolites from diverse chemical classes into their environment. However, we are still only at the beginning to understand the factors that affect the production and the ecological role of these metabolites.

Objectives

Bacterial interspecific interactions are one of the main biotic factors influencing the production of bacterial secondary metabolites (Tyc et al., 2015, 2016). Interestingly many bacteria produce both volatile and soluble secondary metabolites. As compared to soluble compounds, that accumulate around the producing strain rather than diffuse into the environment, volatile compounds are able to diffuse through air- and gas- filled pores and can play an important role in long distance microbial interactions.

Methods

Here we present several examples on the role of soluble and volatile secondary metabolites in microbial interactions as well the importance of inter-specific interactions on bacterial volatile and secondary metabolite production and their antimicrobial activities. Furthermore, we will reveal the importance to study both volatile and soluble metabolites simultaneously and their synergistic effects.

Conclusions

Our studies highlights the importance of interspecific bacterial interactions for triggering secondary metabolites production and the perspectives thereof for extending the range of bioactive compounds to be used for control of plant- and human pathogens.

FEMS7-0207
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EXPRESSION OF DENITRIFICATION GENES IN RESPONSE TO A WATERLOGGING EVENT IN FLUVISOL AND ITS RELATIONSHIP WITH LARGE NITROUS OXIDE PULSES AT DNA AND RNA LEVELS

Y. Uchida[1], Y. Wang[2], H. Akiyama[3], Y. Nakajima[2], M. Hayatsu[2]
[1]Hokkaido University, Research Faculty of Agriculture, Sapporo, Japan
[2]Institute for Agro-Environmental Sciences- National Agriculture and Food Research Organization, Division of Biogeochemical Cycles, Tsukuba, Japan
[3]Institute for Agro-Environmental Sciences- National Agriculture and Food Research Organization, Division of Climate Change, Tsukuba, Japan

Backgrounds

The contributions of large N_2O pulses following waterlogging to the annual cumulative N_2O productions were significant in a Fluvisol. Soil microbial responses to a dry-wet change might be related to the pulse of N_2O gas emissions.

Objectives

To understand the microbial mechanisms underlying these large N_2O pulses in a poorly drained Fluvisol.

To study the effect of soil depth and structure on the large N_2O pulses.

Methods

N_2O emissions were monitored both in intact soil cores and from sieved soils to investigate the importance of soil properties. Quantitative PCR and quantitative RT-PCR analyses were performed to show the correlation among denitrification genes (*nirS*, *nirK*, and *nosZ*) and N_2O emissions at the mRNA level and at the DNA level.

Conclusions

The major pathway for N_2O production was denitrification and larger emissions were observed from the intact soil cores. The change in denitrification gene mRNA levels was more prominent in the 0- to 1-cm soil compared with the 1- to 3-cm soil, in the intact soil cores. Quantitative PCR and quantitative RT-PCR analyses indicated the correlation among denitrification genes (*nirS*, *nirK*, and *nosZ*) and N_2O emissions at the mRNA level but not at the DNA level, particularly at the 0- to 1-cm soil. These indicate that there was a strong variation in soil microbial properties over very small changes in soil depth, and this variation is important in determining the magnitude of N_2O emissions.

FEMS7-0229
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SULFAMETHOXIZOLE/TRIMETHOPRIM-INDUCED ESCHERICHIA COLI O157:H7 TRANSCRIPTIONAL CHANGES FAVOR PROPHAGE-ENCODED VIRULENCE GENES

G. Uhlich[1], E. Andreozzi[1], G. Paoli[1]
[1]Eastern Regional Research Center, USDA/ARS, Wyndmoor, USA

Backgrounds

E. coli serotype O157:H7 genomes contain an abundance of foreign DNA. In the Sakai reference strain, prophage and prophage-like elements comprise nearly 20% of the genome where they control bacteriophage functions, affect host physiology, and regulate virulence. Prophage can also inhibit biofilm formation by inserting in *mlrA*, resulting in truncation of the transcription factor. However, prophage induction can restore *mlrA*, expression of *csgD*, and biofilm formation.

Objectives

We compared RNA-seq profiles of an *E. coli* O157:H7 strain exposed to prophage-inducing concentrations of Sulfamethoxizole/Trimethoprim (SM/T) to investigate biofilm and virulence gene expression.

Methods

RNA collected from O157:H7 strain PA20 grown for either 5 or 12 h on 1% tryptone agar at 30°C ± SM/T at low (20/4 ug/L) and high (540/108 ug/L) concentrations was converted to cDNA and sequenced using Ilumina HiSeq2500.

Conclusions

Results

1. High concentrations of SM/T following 12 h exposure induced ≥4-fold differential expression in >750 genes compared to untreated control

2. Differential expression occurred with higher frequency in prophage, prophage-like, and plasmid genes

3. stx_1, stx_2, and 65% of the genes in the LEE virulence operon were up-regulated

4. High SM/T induced the expression of active truncated forms of *mlrA* by 8-fold; but *csgD* expression showed little changed

Summary

1. At near therapeutic tissue concentrations, SM/T had major effects on O157:H7 gene expression

2. Gene expression changes were common in prophage and plasmid elements

3. Virulence and regulatory genes dominated induced gene populations

4. Truncated *mlrA* products were highly expressed at high SM/T concentrations

FEMS7-1954
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PREVALENCE OF ANTIMICROBIAL RESISTANT MARKER GENES (ARMG) IN A GMO-PRISTINE NORWEGIAN ENVIRONMENT

I. Ullmann[1], H. Tunsjø[2], K.M. Nielsen[3], C. Charnock[1]
[1]Oslo and Akershus University College of Applied Sciences, Life sciences and health- Pharmacy, Oslo, Norway
[2]Oslo and Akershus University College of Applied Sciences, Life sciences and health- Biomedical Sciences, Oslo, Norway
[3]Oslo and Akershus University College of Applied Sciences, Life sciences and health, Oslo, Norway

Backgrounds

Neomycin phosphotransferase II (*nptII)* is one of the most frequently used marker genes in the production of genetically modified organisms (GMO). Its sole function is to aid the production procedure, after which it has no further biological function for the GMO. However, the gene may enter the environment along with plant debris, and potentially transfer to the bacterial soil populations. Coding for an aminoglycoside phosphotransferase enzyme, the *nptII* gene inactivates a range of aminoglycoside antibiotics. Some of these agents, such as neomycin and kanamycin, are critically important antimicrobials in medical and veterinary science (WHO, 2014). If horizontal transfer of intact *nptII* from GM plants to bacteria occurs in the environment, this might interfere with animal and human health by the establishment of resistance to essential antimicrobial agents.

Objectives

The primary objective of this study is to determine the baseline prevalence of *nptII* in the naturally occurring bacterial populations in GMO-pristine agricultural locations in Norway.

Methods

By analyzing sludge samples from water treatment plants (WTP) using an *nptII* specific TaqMan qPCR assay, we will map the copy number of *nptII* from 50 different geographical locations in Norway. The sludge samples will contain bacteria draining from the soils and freshwater areas surrounding each individual WTP.

Conclusions

This will provide a reference, at present lacking, for assessing the effects of aminoglycoside phosphotransferase genes introduced artificially into the environment via GM plants, and the effect this may have on the naturally occurring concentrations of antibiotic resistance determinants in soil and freshwater.

FEMS7-0743
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECT OF LIQUID ORGANIC AMENDMENTS ON SOIL MICROBIAL COMMUNITIES

J. Urra[1], I. Mijangos[1], L. Epelde[1], A. Lanzén[1], F. Blanco[1], C. Garbisu[1]
[1]NEIKER-Tecnalia, Conservation of Natural Resources, Derio, Spain

Backgrounds

The implementation of sustainable agricultural practices is a matter of much interest and investigation. In this respect, fertilization with organic amendments is being used to provide a wide range of nutrients while simultaneously improving the physical, chemical and biological properties of the agricultural soil.

Objectives

Here, we studied the effect of liquid organic amendments (locally called “bioles”), prepared by farmers themselves from organic residues, on soil microbial communities as biological indicators of soil health.

Methods

To this aim, a two-year field experiment was carried out using a randomised block design (n=3) and different doses and forms of application of the abovementioned amendment. After two years, soil samples (0-30 cm depth) were taken to the laboratory where the following physicochemical properties were determined: pH, organic matter, and macro- and micronutrients. Several parameters that provide information on the biomass (microbial biomass C, total bacteria and fungi by qPCR), activity (respiration, enzyme activities, nitrogen mineralization) and diversity (16S amplicon sequencing) of microbial communities were also determined.

Conclusions

Values of corn yield were somewhat lower than those obtained in control plots with inorganic (NPK) fertilizer. However, at a high dose, the application of the organic amendment led to significantly higher values of microbial activity (arylsulphatase and phosphatase activities, nitrogen mineralization) and biomass (total fungi and microbial biomass C). Prokaryotic genetic diversity and soil physicochemical parameters were, in general, not statistically affected by the organic fertilization. It was concluded that these home-made liquid organic amendments have potential for agricultural fertilization and soil health improvement.

FEMS7-1841
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL COMPOSITION OF TECHNOSOLS MADE FROM RECYCLED BENTONITE AND SEWAGE SLUDGE FOR THE REHABILITATION OF A MINING AREA

L. Epelde[1], I. Martín-Sanchez[1], S. Virgel[2], J. Urra[1], A. Lanzén[1], G. Besga[2], C. Garbisu[1]
[1]NEIKER-Tecnalia, Conservation of Natural Resources, Derio, Spain
[2]NEIKER-Tecnalia, Plant Production, Derio, Spain

Backgrounds

Technosols are artificial soils, tailor-made mixtures of wastes that are used to restore degraded areas. However, natural soils contain a myriad of microorganisms that are responsible for many of its vital functions, such as recycling of nutrients, nitrogen fixation, soil structure maintenance, etc. Ideally, one of the objectives of the Technosols should be to correctly fulfill the functions performed by natural soils.

Objectives

To study the microbial composition of Technosols, and compare them to a nearby natural soil.

Methods

The Technosols were established in autumn 2011 at Esther mine (northern Spain) using soil from a waste recovery facility, sewage sludge and recycled bentonite, mixed using three different compositions. The organic matter of each was stabilized in piles for two months before being applied in the field. Triplicate samples from each composition weres then studied and compared to a nearby forest soil as a natural reference. Amplicon sequencing of 16S and 18S rRNA genes was performed for (i) individual ingredients, (ii) the Technosols after stabilization, (iii) the Technosols three years after establishment, and (iv) the reference forest soil.

Conclusions

Out of the ingredients, sewage sludge showed the lowest prokaryotic and eukaryotic diversity. Interestingly, microbial diversity increased significantly in all the Technosols between 2011 and 2014, and its microbial composition was very similar to the reference forest soil in 2014. Therefore, it was concluded that, over time, the microbial communities of the Technosols show signs of resembling the reference soil of the area.

FEMS7-2051
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IDENTIFICATION OF LECTINS THAT BIND AND DIFFERENTIATE COMMON COLIFORMS FOUND IN WATER - APPLICATIONS IN NEXT GENERATION ANALYTICAL PLATFORMS FOR ENVIRONMENTAL SENSING OF BIOLOGICAL CONTAMINATION

S. Deegan[1], M. Utratna[1], N. Islam[1], M. Buscaglia[2], S. Biffi[2], G. Tagliabue[2], A. Bendali[3], I. Pereiro[3], S. Descroix[3], L. Joshi[4]
[1]Aquila Bioscience, Research and Innovation Building, Galway, Ireland
[2]Università degli Studi di Milano, Department of Medical Biotechnology and Translational Medicine, Milan, Italy
[3]Institut Curie, Laboratoire Physico Chimie Curie, Paris, France
[4]Glycoscience Group, National University of Ireland Galway, Galway, Ireland

Backgrounds

Biological contamination of lake water is a serious health risk to the surrounding population of people. If undetected pathogenic bacteria, such as *E. coli* O157:H7, can enter the food chain, wiping out entire animal stocks and ultimately enter our drinking water systems resulting in severe illnesses and potential fatalities.

Objectives

In order to control lake water contamination, routine testing of lake water is conducted; however, currently employed *E. coli* detection procedures often require advanced sample processing involving multiple stages (cell sorting, cell lysing, DNA/RNA extraction and analysis, or petri dish growth methods). These methods are both complex and time consuming and are not suitable for onsite analysis. The next generation analytical platform for environmental sensing (NAPES) will revolutionize onsite water sampling, integrating high speed bacteria detection platforms with state of the art technologies.

Methods

Aquila Bioscience has identified a library of lectins that can capture and detect common water borne bacteria such an *E.coli* O1, *E. cloacae* and *E. coli* O157:H7. The lectin molecules are integrated into a label free RPI detection platform developed by the team at the University of Milano, coupled to the innovative cell sorting method based on immobilized magnetic beads, developed by the team of the Institut Curie (Paris)

Conclusions

The integration of the lectin molecules into the highly sophisticated RPI detection platform and magnetic bead pre-concentration module will allow for a highly specific, highly sensitive and exceptionally fast method for detection of bacteria.

FEMS7-2997
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BIOLOGICAL, MINERAL AND ELEMENTAL STRUCTURE OF EXTANT MICROBIALITES

P. Valdespino[1], P. Hu[2], D. Cerqueda[3], M. Merino-Ibarra[4], L.M. López-Gómez[4], T. Pi-Puig[5], R. González-de-Zayas[6], L.I. Falcón[7], H.Y. Holman[8]
[1]Lawrence Berkeley National Laboratory, EESA- BSISB, Berkeley, USA
[2]Lawrence Berkeley National Laboratory, EESA, Berkeley, USA
[3]Instituto de Ecología- UNAM, Posgrado en Ciencias Biológicas, Mexico City, Mexico
[4]Instituto de Ciencias del Mar y Limnología- UNAM,
Unidad Académica de Ecología y Biodiversidad Acuática, Mexico City, Mexico
[5]Instituto de Geología- UNAM, Lab. Difracción de Rayos-X, Mexico City, Mexico
[6]Centro de Investigaciones de Ecosistemas Costeros, Biogeochemistry, Cayo Coco, Cuba
[7]Instituto de Ecología- UNAM, Lab. Ecología bacteriana, Mexico City, Mexico
[8]Lawrence Berkeley National Laboratory, BSISB, Berkeley, USA

Backgrounds

Microbialites, rock- forming microbial assemblages whose existence extends from the Archaean (~3,500 mya) until present days are useful study models to explore the diversity and the mechanisms involved in carbonates and other minerals precipitation.

Objectives

The role of microbes in the precipitation of minerals needs clarification, the use of emergent strategies of biological and chemical analysis contribute to address questions such as the participation of microbes in mineral formation as well as the chemical and biogeochemical interactions at micro-scale.

Methods

High throughput sequencing, XRD, XRF, SEM-EDS and S-FTIRS Spectromicroscopy were used to asses biological and chemical diversity in a cross-system analysis (5 sampling locations).

Conclusions

Here, we present preliminary results from our studies of the elemental composition of the biomass and the microbialite mineral matrix, and its possible relationships with the microbial community structure in extant microbialites. Regardless their origin (sampling location), microbial groups such as Cyanobacteria, Alpha, Beta and Gammaproteobacteria exhibited the strongest correlations (positive) with organic carbon (Corg), N and particularly with C:S ratio. A diversity of bacterial taxa showed significant (positive and negative) relationships with trace elements (accumulated in microbialite precipitations relatively to their water environments) such as Cd, Co, Cu, Fe, Se and Ni. OTUs exhibiting significant correlations with chemical signatures are shown for biogeochemical parameters, mineral - major ions and trace elements.

FEMS7-0421
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

NATURAL ORGANIC MATTER REDUCTION APPARENTLY FUELS ANAEROBIC OXIDATION OF METHANE IN SEDIMENTS FROM A TROPICAL WETLAND

E.I. Valenzuela[1], A. Prieto-Davó[2], N.E. López-Lozano[3], A. Hernández- Eligio[4], L. Vega-Alvarado[5], K. Juárez[4], A.S. García-González[6], M. G. López[6], F.J. Cervantes[1]
[1]Instituto Potosino de Investigación Científica y Tecnológica, División de Ciencias Ambientales, San Luis Potosí, Mexico
[2]Universidad Nacional Autónoma de México, Facultad de Química- Unidad Sisal, Puerto de Abrigo s/n- Sisal- Yucatán- 97356, Mexico
[3]Instituto Potosino de Investigación Científica y Tecnológica, Ciencias Ambientales, San Luis Potosí- SLP- 78216, Mexico
[4]Universidad Nacional Autónoma de México, Departamento de Ingeniería Celular y Biocatálisis- Campus Morelos, Cuernavaca- Morelos- 62210, Mexico
[5]Universidad Nacional Autónoma de México, Centro de Ciencias Aplicadas y Desarrollo Tecnológico, Coyoacán- D.F.- 04510, Mexico
[6]Centro de Investigación y de Estudios Avanzados del IPN, Departamento de Biotecnología y Bioquímica- Unidad Irapuato, Irapuato, Mexico

Backgrounds

Anaerobic methane oxidation (AOM) avoids the release of huge amounts of this greenhouse gas produced at anoxic zones of water bodies, thus representing a microbial process with great impact on Earth's temperature regulation. Methane is manly emitted naturally by wetlands, despite this, microbial processes involved on its anaerobic consumption on these ecosystems are still poorly understood.

Objectives

The purpose of this study, was to assess the potential of the biota of sediment sampled at a tropical wetland to perform AOM linked to the microbial reduction of its intrinsic electron acceptors, including the electron accepting fraction of natural organic matter (*humus*).

Methods

$^{13}CO_2$ production was documented in microcosms inoculated with wetland sediment supplemented with $^{13}CH_4$ and/or humus. Reduction of sulfate, ferric iron and natural organic matter (NOM)/humus, was followed by capillary electrophoresis and spectrometric methods. Presence and reduction of NOM was qualitatively assessed Micro-ATR-FTIR imaging, UV-Vis-NIR spectroscopy, and XPS. Microbial community composition was investigated by Illumina's 16s Metagenomic Sequencing.

Conclusions

Obtained results showing that NOM partially drives anaerobic methanotrophy, constitutes evidence of a novel process that may largely contribute to supress methane emissions from tropical wetlands. Presence of archaea unrelated to the methane cycle, and absence of well-known methane oxidizers, suggests an unknown role of uncultured archaeal lineages outside the Euryarchaeota phylum involved in AOM.

FEMS7-0535
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DECIPHERING THE FUNCTIONAL CAPACITY OF THE SORGHUM RHIZOSPHERE MICROBIOME

T. Oberholster[1], D. Cowan[1], A. Valverde[1]
[1]University of Pretoria, Genetics, Pretoria, South Africa

Backgrounds

Plant roots interact intimately with rhizospheric microbial communities, which affect both biogeochemical cycling and plant growth. For decades, rhizosphere research has focused on knowing the “who” of microbial communities. As a consequence, very little is known about what these microbes are actually doing. To fill this gap, we investigated the functional potential of the rhizosphere microbiome of an economically important crop in South Africa (i.e., sorghum) grown under field conditions.

Objectives

To elucidate the functional capability of the bacterial communities in the sorghum rhizosphere
To relate the function back to the associated bacterial taxa

Methods

Shotgun metagenomics of 12 samples (6 control + 6 rhizosphere) obtained from 2 soils planted with *Sorghum bicolor*

Conclusions

Our results have shown that sorghum rhizosphere bacterial communities are functionally diverse and different from those of the bulk soil. 425 of the 2552 genes evaluated were more abundant in the rhizosphere than in the bulk soil, whereas 202 genes showed the opposite trend. The rhizosphere soil contained differentially abundant genes involved in the metabolism of carbohydrates, nucleotides, xenobiotics, vitamins and cofactors. In contrast, most of the genes overrepresented in the bulk soil were involved in energy, lipid and amino acid metabolism, as well as in the biosynthesis of glycans, polyketides and terpenoids pathways. These results indicate a difference in the trophic requirements of the rhizosphere microbiome and its response to environmental stressors. We anticipate, that coupling metagenomics of the rhizosphere microbiome with culture-dependent functional analyses will allow us to identify potentially important plant-growth promoting rhizobacteria for sorghum production.

FEMS7-2628
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IMPACT OF FOOD HABITS ON MICROBIOTA PROFILE OF HEALTHY ROMANIAN WOMEN

E. Vamanu[1], *I. Sarbu*[2], *R. Nita*[1], *A. Mircea*[1]
[1]*University of Agronomic Science and Veterinary Medicine, Faculty of Biotechnology, Bucharest, Romania*
[2]*University of Bucharest, Faculty of Biology, Bucharest, Romania*

Backgrounds

Microbial fingerprint of the gut represents a significant aspect in the process of organism adaptation to the environmental changes. Although there is a characteristic microbial fingerprint for each age group or target group with certain food habits, microbiota reacts, by changing its structure, to various bioactive compounds. The capacity to react and to keep favourable strains in a sufficient number will depend on the type and quality of food.

Objectives

The aim of the study was the identification of microbiota fingerprint changes correlated to the presence or absence of meat in the ingested foods.

Methods

Microbiota of minimum three women, for every group, was analysed, because its structure will influence general health. Samples were sterile and kept in glycerol. The women have not received any antibiotic in the last six months. The microbiota reconstitution process was carried out in the GIS1 simulator (www.gissystems.ro) by *in vitro* studies. There were conducted *in vitro* studies and a molecular analysis based on rep-PCR profiles.

Conclusions

With the presence of meat in food habits, it was determined a normal level of favourable strains. These results were correlated with an increase of the ammonium amount in descending colon. Absence of animal protein was correlated with the low level of bifidobacterium strains. The presence of ammonium was similar in all microbiotas. This study allows a better understanding of the role that food nutrients have on the general health of female population. The results can be used to determine a correlation between food habits and organism resistance to infectious diseases.

FEMS7-1179
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ASSESSING THE MICROBIOLOGICAL DIVERSITY IN AQUEOUS ENVIRONMENTS OF A NUCLEAR RESEARCH REACTOR

V. Van Eesbeeck[1], M. Ahmed[1], R. Props[1], R. Van Houdt[1], P. Petit[2], N. Leys[1], J. Armengaud[3], C. Rivasseau[2], J. Mahillon[4], P. Monsieurs[1]
[1]SCK•CEN, Microbiology, Mol, Belgium
[2]CEA, Plant cellular physiology, Grenoble, France
[3]CEA, Biochemistry and Nuclear Toxicology Biochemistry of Perturbed Systems, Bagnols-sur-Cèze, France
[4]UCL, Food microbiology, Louvain-la-Neuve, Belgium

Backgrounds

After being used as energy source inside power plants, spent nuclear fuel must be stored underwater in so-called “spent nuclear fuel pools” (SNFPs) in order to cool down before being safely disposed. Interestingly, despite the highly oligotrophic and radioactive nature of the water, microbial growth is not fully prevented. Microorganisms identified in such environments thus provide a unique opportunity to acquire new insights into survival strategies and radiation-resistance mechanisms.

Objectives

The objective of this work is to inventory the bacterial communities present in SNFPs and other aqueous environments of a nuclear research reactor. In parallel, this research also focuses on following up these communities over time during reactor operation to monitor the long-term effect of ionizing radiation. Finally, this project also aims at a phenotypical characterization of the prevailing species.

Methods

For the inventory and the follow-up of the bacterial communities, a 16S rRNA amplicon sequencing approach was adopted. For the phenotypical characterization, isolates were cultured and their radiation resistance was subsequently tested using a gamma irradiation facility.

Conclusions

Results from the 16S rRNA amplicon sequencing highlighted the presence of bacteria mainly belonging to the Alpha- and Betaproteobacteria. The long-term follow-up of the bacterial communities is still ongoing. The phenotypic characterization showed that all 12 strains tolerated a dose of 300 Gy, but only 8% of the strains was able to cope with a dose of 2100 Gy, indicating large variability in radiation resistance between different strains, and as such not necessarily a high radiation tolerance to survive in these environments.

FEMS7-2543
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ENRICHMENT AND ISOLATION OF NOVEL POLYSACCHARIDE-DEGRADING ANAEROBES FROM ABYSSAL BLACK SEA SEDIMENT

D.M. van Vliet[1,2], *A.J.M. Stams*[1,2,3], *I. Sánchez-Andrea*[1,2]
[1]*Wageningen University, Laboratory of Microbiology, Wageningen, The Netherlands*
[2]*Soehngen Institute of Anaerobic Microbiology, SIAM, Nijmegen, The Netherlands*
[3]*University of Minho, Centre of Biological Engineering, Braga, Portugal*

Backgrounds

Anoxic marine sediments form an important global carbon sink. However, we know little about the ecophysiology of the dominant organic matter-degrading anaerobes from this habitat, due to the lack of ecologically relevant anaerobic isolates. In contrast, important marine aerobes such as SAR11 bacteria were isolated succesfully with oligotrophic, low-nutrient media. Young anoxic sediments are not poor in nutrients, but important anaerobes could nevertheless be oligotrophs, because of the recalcitrance of available biopolymer substrates such as polysaccharides.

Objectives

We aim to enrich and isolate ecologically relevant marine anaerobes from the Black Sea under environmentally representative conditions with polysaccharides as substrates.

Methods

As inoculum, we used anoxic Black Sea sediment from the abyssal plain at 2100 m depth. We set up enrichment cultures with mineral marine medium and with several polysaccharides as separate substrates. The cultures were incubated at 15 °C. From these enrichments, physiologically novel strains of *Psychromonas* and *Marinifilum* were isolated. A previous stable isotope probing experiment with ^{13}C-glucose supports the ecological importance of an obtained *Psychromonas* strain in sediments. Analysis of 16S rRNA gene amplicons revealed that in some cultures the uncultivated *Lentisphaerae* R76-B128 clade was enriched, which is our current focus of isolation. This clade has been found in anoxic marine waters, including those of the Black Sea. The most closely related isolate (89% 16S rRNA identity) is a halophilic, saccharolytic oligotroph.

Conclusions

By cultivation under environmentally representative conditions with polysaccharides as substrates, we enriched and isolated ecophysiologically relevant marine anaerobes.

FEMS7-3124
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SOLUBILIZATION OF PHOSPHORUS AND PLANT GROWTH PROMOTION BY ACTINOBACTERIA IN SOYBEAN CROP

H. Vargas Hoyos[1], *J. Chiaramonte*[1], *I. Soares de Melo*[2]
[1]*University Of Sao Paulo / Luiz de Queiroz College of Agriculture, Soil Biology, Piracicaba, Brazil*
[2]*Embrapa / Envtironment, Environmental Microbiology, Jaguariúna, Brazil*

Backgrounds

Several studies have already investigated the Actinobacteria as source of raw material for drug discovery. Not less important is the function that they develop in the nutrients cycling making then available for plants. The “3AS4” *Streptomyces rishiriensis* isolate was selected due to its exceptional phosphorus solubilization.

Objectives

The aim of this study was to investigate the possible mechanism of action involved in phosphorus solubilization and access the plant growth promotion ability of this strain.

Methods

In order to recognize its mechanism of action, the crude extract was monitored during 10 days with HPLC. A greenhouse experiment was carried out to evaluate the potential plant growth promotion of the isolate in soybean. Besides the soil with no phosphorous, two phosphate sources: Triple super phosphate (TSP) and Rock phosphate Bayovar (RPB) were added at the soil. Inoculated pots were compared to control pots in the three concentrations of phosphates (0, 20 and 40 Kg/h). Evaluations considered the plants attributes; chlorophyll relative index, height, dry shoot and root biomass. The crude extract presented a chromatographic profile similar to gluconic acid. Significant differences in plant height were observed at 8 weeks when inoculated with the isolate. The Shoot/Root index was significantly higher when applied the higher dose of RPB along with *S. rishiriensis*. When inoculated with the isolate, the plants with non-additional phosphorous showed similar development compared to the control pots with 40 Kg/h of TSP and RPB.

Conclusions

These results suggest that Actinobacteria might be a valuable resource for sustainable agriculture application by reducing phosphate fertilization.

FEMS7-2071
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

IDENTIFYING GENOME-WIDE GENETIC VARIATION IN POPULATIONS OF BACTERIAL ENDOSYMBIONTS OF INSECTS

C. Vargas-Chavez[1], *A. Latorre*[1]
[1]*Institute for Integrative Systems Biology I2SysBio, Evolutionary Systems Biology of Symbionts, Paterna, Spain*

Backgrounds

Bacterial symbionts are widespread in insects. This has allowed insects to thrive on unfavorable environments and exploit previously inadequate food sources, such as the carbohydrate-rich but amino acid-poor plant sap. Many of these symbionts are harbored inside specialized cells called bacteriocytes. In this constant and isolated environment, the bacterium reduces its functions (genes) to the extreme conserving only those that are relevant to its host. In some cases, more than one endosymbiotic bacteria may reside in the same host, such as the *Buchnera-Serratia* symbiotic consortium in some Lachninae aphids like *Cinara cedri*. The usual method of transmission is vertical, with offspring inheriting the symbionts directly from their mothers. This method of inheritance subjects the bacteria to strong bottlenecks each insect generation.

Objectives

We are interested in detecting the effects this bottleneck has on the diversity of the bacterial endosymbionts population residing in an insect. Our main goal is identifying variation in the population, such as SNPs and indels and if possible identify haplotypes.

Methods

High-quality assemblies from endosymbiotic bacteria of different genus were used as references. Next-Generation Sequencing reads were mapped to these assemblies and SNPs and indels were identified and their frequencies in the population were quantified. The phenotypic effect of each variant was determined and compared among and between members of the different genus used.

Conclusions

Regardless of the bottlenecks the endosymbiotic bacteria are subjected to, it is possible to identify variants in all members evaluated. Some trends on the variation rates and their consequences for the bacterium were identified.

FEMS7-0736
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

TESTING THE HYPOTHESIS OF ACQUIRED AMINOGLYCOSIDE RESISTANCE IN RALSTONIA SPP.

I. Vaz-Moreira[1,2], P. Ferro[1], O.C. Nunes[2], C.M. Manaia[1]
[1]Escola Superior de Biotecnologia - Universidade Católica Portuguesa/Porto,
CBQF – Centro de Biotecnologia e Química Fina, Porto, Portugal
[2]Faculdade de Engenharia- Universidade do Porto,
LEPABE - Laboratório de Engenharia de Processos- Ambiente- Biotecnologia e Energia, Porto, Portugal

Backgrounds

Ralstonia spp. are ubiquitous in water environments, including drinking water. The species *R. pickettii* and *R. mannitolilytica* have been associated to nosocomial outbreaks and to a wide array of antibiotic resistance phenotypes. Aminoglycoside resistance (AGR) is a variable phenotype in those *Ralstonia* species, suggesting that it can be acquired and not intrinsic as, for example, colistin resistance.

Objectives

Investigate the AGR mechanisms in *Ralstonia* spp. isolated from aquatic environments and assess a possible relationship with the type of water (mineral, tap, wastewater) from which they were isolated. Search hints for a possible AGR acquisition.

Methods

A collection of >50 *Ralstonia* spp. (including some successively transferred in the presence of gentamicin) were characterized for their AGR phenotypes. From this collection two *R. pickettii*, with distinct AGR phenotype and isolated from the same habitat (hospital effluent), were selected for comparative genome sequencing analysis. In this analysis, genetic determinants such as ICEs (integrative conjugative elements), efflux pumps and 16S rRNA methylases that could be worthwhile to screen in the *Ralstonia* spp. were selected.

Conclusions

Beside AGR, resistance to beta-lactams and colistin was frequent in *Ralstonia* spp., regardless the type of water. The comparative whole genome analysis revealed that genes related with tolerance to arsenic and toxic compounds, ICEs, lysozyme inhibitors and with phages/prophages were only present in the AGR strain. Whilst it was not possible to identify AGR determinants associated with ICEs, components of these genes cassettes were detected in most of the AGR strains, leading to the hypothesis that ICEs may be associated with genome dynamics mechanisms. AGR mechanisms through efflux pumps and/or 16S rRNA methylases are being explored in these strains.

FEMS7-1894
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BACTERIAL COMMUNITY AND ANTIBIOTIC RESISTANCE DYNAMICS IN A FULL-SCALE WASTEWATER TREATEMENT PLANT WITH UV DISINFECTION

C. Narciso-da-Rocha[1], J. Rocha[1], V.M. Ivone[1], F. Lira[2], J. Tamames[2], I. Henriques[3], J.L. Martínez[2], C.M. Manaia[1]
[1]Universidade Católica Portuguesa - Escola Superior de Biotecnologia,
CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Porto, Portugal
[2]Consejo Superior de Investigaciones Científicas - CSIC, Centro Nacional de Biotecnología, Cantoblanco- Madrid, Spain
[3]CESAM - University of Aveiro, Biology Department, Aveiro, Portugal

Backgrounds

It has been proposed that wastewater treatment plant can be reservoirs and reactors of antibiotic resistance. Since wastewater treatment lead to variations on the bacterial community composition, knowing how such variations impact the dynamics of antibiotic resistance genes (ARGs) is critical to improve ARGs removal during wastewater treatment.

Objectives

To study an urban wastewater treatment plant (UWTP) which includes secondary and UV treatment aiming at: i) identifying populations more fitted during the whole process; ii) measure the impact of each treatment step on the abundance of ARGs; iii) infer how population changes may alter the distribution of ARGs.

Methods

Samples were collected at three dates from the raw inflow, secondary (activated sludge) and tertiary (UV disinfection) effluent of a UWTP. The cultivable enterobacteria, bacterial community composition and 8 ARGs were examined in all samples and after three days storage.

Conclusions

Each of the stages of activated sludge and UV disinfection led to ~2 log-units reductions of enterobacteria. Noteworthy it was the secondary treatment, rather than UV, that led to important changes in the bacterial community composition. In addition, quantitative PCR of ARGs, showed reduction of ~2 log-units after the secondary treatment and a negligible variation after UV disinfection. The effect of treatment was not identical for all ARGs examined, an observation that was consistent with the fact that different genes were most correlated with distinct bacterial populations. For instance, members of *Bacteroidaceae*, *Lachnospiraceae*, *Campylobacteraceae, Aeromonadaceae*, *Enterobacteriaceae* and *Moraxellaceae* were correlated with beta-lactamase and *qnrS* genes, while members of *Comamonadaceae*, *Neisseriaceae* and the classes *TM7-1* and *ZB2* were correlated with the gene *sul2*.

FEMS7-2782
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

METABOLISM OF SOY ISOFLAVONES BY BACTERIA OF INTESTINAL ORIGIN

L. Vázquez[1], *A.B. Flórez*[1], *B. Mayo*[1]
[1]*IPLA-CSIC, Microbiología y Bioquímica de Productos Lácteos., Villaviciosa-Asturias, Spain*

Backgrounds

High intake of soy or purified isoflavones has been associated with less intense menopause symptoms and a reduced risk of hormone-mediated diseases. Isoflavones from the diet can be metabolized into fully-active compounds or inactive metabolites by bacteria from the human gastrointestinal tract, which at present are poorly characterized.

Objectives

In this work, we assessed the metabolism of the two majority soy isoflavones (daidzein and genistein) by cultures of faecal slurries from menopausal woman with a positive or negative equol-production phenotype.

Methods

Faecal samples of five women were homogenized and inoculated in a general medium for colon bacteria. Faecal homogenates, bulk cultures and isolated colonies from the counting plates were incubated in GAM+0.5% arginine supplemented with 100 µM daidzein or genistein. Isoflavones and their metabolites in the cultures were analysed by UHPLC. In addition, microbial DNA was purified and used in conventional and real-time PCR analysis in order to identify and quantify specific genes involved in equol formation from daidzein.

Conclusions

Equol was only present in cultures derived from equol-producing women inoculated with homogenates and bulk colonies from the counting plates. Variable amounts of genistein and dihydrogenistein were scored when this isoflavone was added. Both *ddr* and *tdr* genes, encoding reductases involved in equol production, were detected in similar amounts in DNA from faeces and cultures thereof of equol-producing women. Occasionally, these genes were also correlated with genistein-derived metabolites. The biological significance of the presence/absence of *tdr* and *ddr* in isoflavone metabolism is currently under study.

FEMS7-2118
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ENDOPHYTIC MICROORGANISMS ISOLATED FROM GREEK VARIETY OF SOLANUM LYCOPERSICUM, ENDEMIC MEDICINAL PLANTS AND OLEA EUROPAEA AS BIOSTIMULATING AND/OR BIOCONTROL AGENTS AGAINST PLANT PATHOGENS

K. Nifakos[1], D. Douka[2], T.N. Spantidos[2], I.E. Thomloudi[2], A. Davari[2], P. Tsalgatidou[2], M. Dimou[2], A. Venieraki[2], C. Delis[1], P. Katinakis[2]
[1]Technological Educational Institute of Peloponnese- School of Agricultural Technology and Food Tec hnology and Nutrition-, Agricultural Technology, Kalamata, Greece
[2]Agricultural University of Athens,
Laboratory of General and Agricultural Microbiology- Dept. of Crop Science, ATHENS, Greece

Backgrounds

It is possible to increase and improve the agricultural productivity by stimulating plant yield and protecting crops from phytopathogens. Commercial synthetic fertilizers and pesticides are insecure for public health. Farmers are encouraged to use environmentally friendly alternatives. Biofertilizers and biopesticides may become the preferred substitutions for some conventional synthetic products.

Endophytes colonize an ecological niche similar to that of phytopathogens, which make them candidate for disease suppression.

Objectives

We aim to isolate endophytic bacterial and fungal strains from a Greek (Messinian) variety of Solanum lycopersicum “Chontrokatsari”, greek medicinal plants (Salvia spp., Teucrium spp., Hypericum spp.), and olive tree Olea europaea. In parallel, we investigate and characterize the antifungal, antibacterial activity and other beneficial traits of the endophytic isolates and we test the most promising isolates for their ability to promote plant growth and to antagonist against phytopathogens.

Methods

Isolation of endophytes from leaves, stems, roots and seeds. Beneficial and biocontrol traits such as: phosphate solubilisation, siderophore, IAA, chitinase production, Swarming motility, biofilm formation, NRPs production etc. of the isolated strains.

The antimicrobial activity was confirmed by the visualization and measurement of any inhibition zones.

Conclusions

This is the first report of the endophytic microbial communities that colonize the phyllosphere, stem and seed endo-sphere of Solanum lycopersicum ‘Chontrokatsari’, olive tree plant, and the aforementioned Greekmedicinal plant. Additional greenhouse and field experiments are required to provide more conclusive information about the potential of using endophytic beneficial strains for plant inoculation in soil with limited nutrient resources, for example in combination with different beneficial bacteria.

FEMS7-1817
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL COMMUNITY COMPOSITION AND DIVERSITY OF A LARGE-SCALE SOUTH AFRICAN DRINKING WATER DISTRIBUTION SYSTEM

S. MacRae[1], A. Pinto[2], M. Sigudu[3], N. Esper[3], H. du Preez[3], S. Venter[1]
[1]University of Pretoria, Microbiology and Plant Pathology, Pretoria, South Africa
[2]Northeastern University, Civil and environmental Engineering, Boston, USA
[3]Rand Water, Scientific Services, Vereeniging, South Africa

Backgrounds

Microbial processes play a crucial role in the production and distruibution of safe drinking water. Long-term investigations into drinking water distribution systems can reveal robust processes, infrastructure, and environmental factors that influence the microbial community, offering opportunities to re-think microbial control in drinking water systems.

Objectives

In this 2-year study, we assessed the bacterial community composition and diversity in a large, full scale South African drinking water distribution that uses both chlorine and chloramine as primary and secondary disinfectants, respectively.

Methods

Bulk water samples were collected on a monthly basis at the outlet of the treatment plant and at 17 points in the distribution system spanning nearly 150 kilometres. Illumina Miseq sequencing of the V4 hypervariable region of the 16S rRNA gene revealed a highly diverse bacterial community consisting of 9,756 operational taxonomic units.

Conclusions

Similar to other studies, *Alpha*- and *Betaproteobacteria* dominated the drinking water bacterial communities, with an increase in *Betaproteobacteria* post-chloramination. Temporal variation was consistently stronger than the spatial changes and demonstrated seasonal cycling, which correlated with changes in the temperature. The observed richness, diversity, and evenness of the bacterial communities were higher in the winter months as opposed to the summer months. The bacterial communities also showed distance decay features, with bacterial communities becoming increasingly dissimilar with increasing distance between sampling locations. This study emphasises the significance of a long-term study to fully understand how environmental factors as well as the microbial community within the system could affect the bulk water ecosystem.

FEMS7-1071
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHARACTERIZATION OF THE MICROBIAL COMMUNITY FROM HYPERSALINE SOILS AND ITS METABOLIC POTENTIAL

B. Vera-Gargallo[1], *C. Sánchez-Porro*[1], *A. Ventosa*[1]
[1]*University of Sevilla, Microbiology and Parasitology, Sevilla, Spain*

Backgrounds

Hypersaline environments are extreme habitats in which life is limited by a low water activity due primarily to high concentrations of sodium chloride. These particular conditions can be found, among others, in hypersaline lakes, solar salterns, salt-curated food and saline soils. While the microbial communities from aquatic systems have been extensively studied, hypersaline soils have received much less attention.

Objectives

To determine the genomic diversity of hypersaline soils from the Odiel saltmarshes, in the Southwest of Spain, comparing the results with those from solar salterns, from which extensive data exist.

Methods

Two 454 shotgun metagenomes from unvegetated soils located in Odiel saltmarshes were obtained in different years. Quality filtering of the datasets was performed with Prinseq, CDSs were predicted with Prodigal. Newbler was employed for assembly. Taxonomic assignments of the contigs was performed with BLAST+ and MEGAN. Paprika and PathwayTools allowed the analysis of the metabolic potential. MetaBAT, VizBin and CheckM were used for obtaining and curating bins.

Conclusions

At the phylum level, the studied soils are similar to salterns crystallizer ponds, in which the dominant phylum is *Euryarchaeota*, being *Bacteroidetes*, *Betaproteobacteria* and *Actinobacteria* the most represented bacterial phyla. However, we did not find sequences related to the genus *Haloquadratum*. Interestingly, the most complete bin obtained was related to *Sphingobacteriales* and not *Euryarchaeota*, which could be due to the high intra-genus heterogeneity of the organisms from the latter taxon. The phylum *Nanohaloarchaeota* is also present. The metabolic potential of these phyla and the obtained metagenomic bin has also been addressed.

FEMS7-1110
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

FACTORS INFLUENCING THE MICROBIAL COMMUNITY STRUCTURE OF SALINE SOILS FROM THE ODIEL SALTMARSHES

B. Vera-Gargallo[1], T. Roy-Chowdhury[2], J. Brown[2], C. Sánchez-Porro[1], V. Bailey[2], J.K. Jansson[2], A. Ventosa[1]
[1]University of Sevilla, Microbiology and Parasitology, Sevilla, Spain
[2]Pacific National Northwest Laboratory, Biological Sciences Division, Richland, USA

Backgrounds

Studies of saline and non-saline environmental habitats have shown that salinity is a main factor driving the assemblage of microbial communities. However, it is unknown to what extent osmotic stress affects microbial community structure within a set of saline samples. In this study we investigated the relative influence of salinity, metal content and other physicochemical factors on the distribution of taxa in soil samples having a range of salinities.

Objectives

To assess the relationship of salinity, metal content, and other soil properties on the structure of the bacterial and archaeal communities dwelling in saline soils from the Odiel saltmarshes (Southwest Spain).

Methods

Sequencing of 16S rRNA gene amplicons was performed according to the protocols of the Earth Microbiome Project for soil samples from four sites (referred to as Sites 1 to 4) and two depths (0-1 cm and 1-5 cm). The salinity of these samples ranged from 8.2 – 50.1 dS m^{-1}.

Conclusions

Across the range of salinities studied, the main factor influencing the variability in microbial communities was the sampling site and not the level of salinity. This may suggest that all of these samples surpassed a critical threshold of salinity requiring all microorganisms present to be highly adapted and that under these conditions other physicochemical characteristics of the sites were more significant in structuring the microbial community present. Texture, water content and calcium, aluminum, sulfate, sulfur and phosphorous concentration were significant features explaining the variability between the saline soils samples studied.

FEMS7-1647
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION AND DETERMINATION OF NOVEL PLASMIDS FROM BACTERIA OF HONEY AND HONEY STOMACH FLORA

A. Veress[1], *J. Kömüves*[1], *F. Olasz*[1], *J. Kiss*[1]
[1]*NARIC-Agricultural Biotechnology Institute, Department for Genetics, Gödöllő, Hungary*

Backgrounds

Several lactic acid bacteria (LAB) have positive effects on human and animal health, thus they are utilized as probiotics. LAB can be isolated from many sources such as gut flora of vertebrates or insects.

Objectives

The aim of our project was to isolate LAB strains for probiotic use from honey and honey stomach of *Apis mellifera*, however, many other species have also been found. These new yet undescribed strains can be the sources of previously unknown plasmids potentially harbouring new genes of scientific interest.

Methods

According to 16S rDNA-amplicon profiling and restriction analysis of total DNAs indicating the presence of plasmids, three strains have been selected for whole genome sequencing. Bioinformatics analysis revealed the presence of eight *rep*A genes (encoding replication proteins of plasmids) in the three strains, which were classified into *Lysinibacillus*, *Saccharibacter* and *Acinetobacter* genera. Seven *rep*A genes were located on scaffolds that could be converted into circular plasmid sequences, while the last one appeared to belong to a large plasmid of >170 kb. Furthermore, an 8-kb *rep*A-less plasmid could also be identified by restriction analyses.

Conclusions

Altogether, seven novel complete plasmid sequences and one already known plasmid have been validated by PCR, while scaffolds of the >170 kb unnamed *Lysinibacillus* plasmid have to be determined. To date, pAV*Aci*147 and pAV*Lys*47 have been cloned and transferred into *E. coli* cells and the isolation of pAV*Aci*84, pAV*Aci*117, pAV*Sac*7 and pAV*Sac*8 is ongoing. The 45 kb pAV*Aci*14 appears to have a complete set of conjugation genes, thus its mobilization is also in progress.

FEMS7-1953
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DETECTION OF HELICOBACTER PYLORI BY PCR OF THE GLMM GENE IN SURFACE WATER FROM BOGOTA (COLOMBIA)

F.J. Vesga Pérez[1,2], Y. Moreno[2], M.A. Ferrús[3], C. Campos[1], A.A. Trespalacios[1]
[1]Pontificia Universidad Javeriana, Microbiology Department- Science Faculty, Bogotá, Colombia
[2]Universitat Politécnica de València, Research Institute of Water Engineering and Environment IIAMA, Valencia, Spain
[3]Universitat Politécnica de València, Biotechnology Department. ETSIAM, Valencia, Spain

Backgrounds

Helicobacter pylori causes gastric diseases and peptic ulcers and plays an important role in gastric cancer and lymphoma, classified by IARC-WHO as a class I carcinogen. It has been detected in different water systems around the world, but few studies have reported successful culture of *H. pylori* from water. Thus, application of molecular methods for rapid, sensitive, and specific detection of *H. pylori* in aquatic environments is of the most importance.

The *glmM* gene is essential for the growth of the microorganism, and it has been used for confirming the presence of *H. pylori* in waters.

Objectives

The aim of this work was to determine the occurrence of *H. pylori* by polymerase chain reaction (PCR) of the *glm*M gene in surface water from San Rafael reservoir (Bogotá, Colombia).

Methods

Fifty surface water samples were collected, centrifuged at 4,000rpm/20min and resuspended in PBS buffer. The suspension was subjected to DNA extraction with the DNeasy Blood & Tissue (Qiagen) kit. PCR for detection of a fragment of 132 base pairs of the conserved region of the glmM gene of *H. pylori* was performed.

Conclusions

The overall detection rates for *H. pylori* DNA in the samples were 32% (16/50). In conclusion, the detection of *H. pylori* at different sampling times indicate either a continual contamination source or persistence of *H. pylori* in surface water.

This study has been supported by Colciencias, convocation #569-2012, and collaboration of Aqueduct and sewage company of Bogotá (EAB) of Colombia and Spanish Ministry of Economy and Competitiveness AGL2014/53875-R grant.

FEMS7-2328
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SPECIES-FUNCTION RELATIONSHIPS SHAPE ECOLOGICAL PROPERTIES OF THE HUMAN GUT MICROBIOME

S. Vieira-Silva[1], G. Falony[1], J. Raes[1]
[1]KU Leuven, O&N Rega, Leuven, Belgium

Backgrounds

The colon microbial ecosystem is characterized by a shallow phylogenetic diversity within only a few deep lineages. Within the microbiota, functional repartition is believed to be driven both by microbial competition and niche specialization and by host selective pressure towards commensalism and functional redundancy. Studying the microbiota complementarity patterns and resulting intricate food webs is now made easier through metagenomic analysis.

Objectives

The main objective is to assess how species-function relationships in the gut microbiota can be linked to gut bacteria fitness and to ecosystem resilience.

Methods

We explore saccharolytic/proteolytic/lipolytic fermentation potential in the human gut using a novel manually curated gut-specific metabolic module framework, made available as a public resource. We first use these metabolic modules on gut reference genomes to identify species metabolic specialization into these 3 main fermentation axes or generalist strategies. We later use these on sequenced gut metagenomes to study ecosystem functional redundancy comparing metagenomes as classifed into enterotypes, and link it to microbial growth rates.

Conclusions

We observe genus-level metabolic specialization, mostly achieved through input-level adaptation. Notably, proteolysis specialization is negatively correlated to capacity for fast growth. Genus-level metabolic consistency varies significantly, being low in Firmicutes genera and higher in *Bacteroides*. Ecosystem-level variation between individuals is also shaped by metabolic preference for specific fermentable substrates, as each enterotype shows distinct saccharolytic/proteolytic/lipolytic profiles. Finally, we find that functional redundancy between gut taxa is reduced in low-richness, *Bacteroides*-type individuals, indicating a potential decreased resilience to perturbation, in line with their frequent association to dysbiosis.

FEMS7-2014
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

DISENTANGLING MICROBIAL COMMUNITIES IN THE SOUTHERN OCEAN USING METAGENOME BASED GENOME RECONSTRUCTIONS

S. Vikram[1], O. Bezuidt[2], S. Phoma[1], D. Cowan[1], T. Makhalanyane[1]
[1]University of Pretoria, Centre for Microbial Ecology and Genomics, Pretoria, South Africa
[2]Agricultural Research Council, Biotechnology Platform, Pretoria, South Africa

Backgrounds

Understanding how oceanographic features shape marine microbial ecosystems and their implications on biogeochemical cycling remains a major ecological endeavor. Recently, several studies have assessed the contribution of photoautotrophs in surface waters and the factors which shape them. However, in contrast very little is known regarding chemolithoautotrophs in marine ecosystems generally and waters south of 40^{o}.

Objectives

Here, we assess microbial diversity, and functional capacity along the Agulhas current system and the Subtropical convergence in the South Indian Ocean.

Methods

Samples collected from three water columns, epipelagic zone, oxygen minimum zone (OMZ) and bathypelagic zone, were analysed using shotgun metagenomics and bionformatics tools.

Conclusions

We found high taxonomic richness in surface and deep water sample members, with generally low numbers for middle samples, corresponding to the oxygen minimum zones, in contrast to previous studies. Community analysis revealed significant dissimilarity between the three water depths; dominated by marine Proteobacteria, followed by Bacteroidetes, Actinobacteria, and Firmicutes with strikingly low cyanobacterial abundance. Our data showed evidence of extensive carbon, nitrogen and sulphur biogeochemical cycling capacity with a large proportion of functional genes belonging to Alphaproteobacteria (Rhizobiales), Gammaproteobacteria (genus Pseudoalteromonas) and Cyanobacteria (genus Synechococcus). We have reconstructed the bacterial genome bins. The most completed genome bin shows highest similarity to the genus *Psychrobacter*, having genes for the sulfur and iron metabolism, suitable for the South Indian Ocean marine environment. Taken together, our results suggest differential microbial community structure along with the water columns. Functional analysis revealed a variety of traits driven by these taxa together with Proteobacteria, which suggests high functional redundancy.

FEMS7-0564
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

CHARACTERIZATION AND IDENTIFICATION OF A BACTERIUM WITH HIGH TOLERANCE TO CHROMIUM(III) ISOLATED FROM A MICROALGA CONSORTIUM OF EBRO DELTA MICROBIAL MATS

E. Villagrasa[1], N. Ferrer-miralles[2], L. Millach[1], A. Obiol[1], J. Creus[1], I. Esteve[1], A. Sole[1]
[1]Universitat Autònoma de Barcelona, MICROBIOLOGIA, BELLATERRA, Spain
[2]Institut de Biotecnologia i de Biomedicina, Microbiologia Aplicada, BELLATERRA, Spain

Backgrounds

In the last years, our working group has isolated, from Ebro delta microbial mats, a heterotrophic microorganism (*Ochrobactrum* sp. DE2010) belonging to a microalga consortium. Although, the effect of different metals on this consortium has been investigated, very little is known about the metals effect on this heterotrophic microorganism. In this study chromium(III) has been selected because is considered as a micronutrient, but at high concentrations it can be an important pollutant with toxic effects.

Objectives

Characterize and identify the heterotrophic microorganism. Evaluate the cytotoxic effect of chromium(III) on this bacterium and its capacity to remove it.

Methods

For the first objective, morphological biochemical and physiological analysis and its phylogenetical identification were realized. To evaluate the cytotoxic effect of Cr(III) the changes in biomass/viability of this microorganism were determined by the fluorochrome-CLSM-Image Analysis method. Moreover, its capacity to accumulate Cr(III) extra- and/or intracellularly was evaluated by STEM-EDX, and its uptake efficiency of removal Cr(III) and the specific metal removal (q) were determined by ICP-OES. Finally, changes in its EPS composition were also monitored using different physico-chemical and spectrophotometric methods.

Conclusions

The new microorganism was tentatively identified as *Ochrobactrum* sp. DE2010 and it could be seen capable of restoring polluted environments by chromium(III), since it is: indigenous in Ebro delta microbial mats, an ecosystem polluted by this metal; easy to grow in axenic cultures; able to absorb Cr(III) in EPS and in polyphosphate inclusions and exhibits a great tolerance to and a high removal affinity for chromium(III).

FEMS7-2668
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INTERACTIONS OF POLYPORALES SAPROTROPHIC FUNGI AND EFFECT ON WOOD-DECAY ENZYME ACTIVITIES

T. Virolainen[1], J. Kuuskeri[1], H. Mattila[1], F. Shah[1], T. Lundell[1]
[1]Department of Food and Environmental Sciences, University Of Helsinki, Helsinki, Finland

Fungal communities are dynamic in nature. Fungal species and isolates may be tolerant or weak antagonists, or even strong combatants upon interactions. The influence of *Fomitopsis pinicola*, a common brown-rot Basidiomycota species of Polyporales, on the growth and enzyme production of the Polyporales white rot species *Phlebia radiata* and *Trichaptum abietinum* was studied in co-cultivations and on coniferous wood including medium. Production profiles of activities of CAZymes and oxidoreductases (laccase, manganese peroxidase, xylanase, beta-glucosidase) together with enzymes involved in recycling of organic nitrogen and hyphal decomposition were studied. In fungal combination cultures on solid agar media, *F. pinicola* was a supreme colonizer quickly advancing over hyphae of the white rot species. Mycelial blocks against the other white rot species were generated by *P. radiata* but not against *F. pinicola*. In most of the wood-supplemented cultures, fungal production of oxalic acid acidified the medium, which was in correlation to generation of fungal biomass. In solid-state cultures on spruce wood sawdust, wood substrate consumption and mass loss occurred as decrease of total dry weight. Our results indicate that fungal interactions have an outstanding role in wood-degradation processes and carbon cycling in the forest ecosystems.

FEMS7-0430
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

SPATIAL VARIATION OF ENZYME ACTIVITY AND ORGANIC CONTENT IN SOIL TO ASSESS CROP YIELD ALONG A LATITUDINAL GRADIENT IN NORTH-CENTRAL INDIA

K. Vishwakarma[1], N. Kumar[1], N. Upadhyay[1], S. Sharma[1]
[1]Motilal Nehru National Institute of Technology Allahabad, Department of Biotechnology, Allahabad, India

Backgrounds

Soil is the incoherent matter on Earth's surface having organic and mineral content. Soil health depends on its soil enzymes which mainly originate from soil microbes that indicate microbial activity in soil environment and play an important role in organic matter decomposition. The decomposition helps in nutrient cycling and regulation of soil fertility. This study was facilitated to encompass the effect of climate change, enzyme activity on nutrient cycling i.e. organic carbon, available nitrogen and phosphorous in respective agro-ecosystems.

Objectives

1. To isolate and characterize bacteria from soil of different regions along the latitude gradient in north central India.
2. To analyze the soil enzyme activities and microbial biomass in order to assess soil quality.

Methods

Top soils were sampled in fields at four locations namely, Solan (Himachal Pradesh), Jagatpura (Punjab), Allahabad (Uttar Pradesh) and Satna (Madhya Pradesh). Enzyme activities including dehydrogenase, polyphenol oxidase, urease and catalase along with microbial biomass (nitrogen and carbon) were assessed along the latitudinal gradient. Analysis of soil biological and chemical properties was also done.

Conclusions

With increasing latitude, organic carbon decreases whereas available phosphorous and available nitrogen increases ($P<0.05$). With increase in the latitude, polyphenol activity and urease activity decreases, dehydrogenase activity increases whereas catalase activity showed no significant relation with latitude ($P<0.05$). This distribution of enzyme activities was consequence of alterations in temperature and moisture of soil because of which soil properties changes along the latitudinal transect.

FEMS7-0431
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM INDIAN MUSTARD (BRASSICA SPP.)

K. Vishwakarma[1], *N. Upadhyay*[1], *N. Kumar*[1], *S. Sharma*[1]
[1]*Motilal Nehru National Institute of Technology Allahabad, Department of Biotechnology, Allahabad, India*

Backgrounds

Rapeseed-Mustard i.e. *Brassica* spp. is 3rd most important oil-seed crop in the world after soybean and palm. In India, it is second most important edible oilseed after groundnut sharing 27.8% in India's oil-seed economy. To enhance the production and yield of *Brassica* in middle Indo-gangetic plain region of Uttar Pradesh i.e. Allahabad, the plant growth promotion attributes were assessed.

Objectives

1. To isolate and characterize the plant growth promoting rhizobacteria from soils of different regions in Allahabad.
2. To analyze the soil enzyme activities and microbial biomass in order to assess soil quality.

Methods

Bacterial isolates were isolated from the soil samples of *Brassica spp.* taken in triplicates from three different regions by serial dilution method. The soils collected from different regions were also tested for quality by assessing soil enzyme activities (dehydrogenase, polyphenol oxidase, urease and catalase). The isolates were characterized on the basis of their morphological and biochemical characteristics. Furthermore, isolates were screened for plant growth promoting potential *viz.* phosphate solubilization, free nitrogen assimilation, hydrogen cyanide (HCN), indole 3-acetic acid (IAA) and siderophore production.

Conclusions

On the whole, twenty-two bacterial isolates were identified on the basis of their abundance in respective regions. Out of these twenty-two isolates, seven showed plant growth promoting activities. Future studies will incorporate the utilization of potential isolates in plant growth promotion *in vitro*.

FEMS7-2433
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INSIDE THE SEED MICROBIOME: FUNGAL AND BACTERIAL SEED ENDOPHYTES IN PLANT RESPONSES TO CLIMATE CHANGE

V. Vujanovic[1], J.J. Germida[2]
[1]University of Saskatchewan, Food and Bioproduct Sciences, Saskatoon, Canada
[2]University of Saskatchewan, Soil Science, Saskatoon, Canada

Backgrounds

Recent studies have found that mycovitality and bactovitaity or the endosymbiotic seed-microbiomes relationship bear tangible benefits for sustainable agriculture production.

Objectives

The aim of the study is to explore the role of endophytes as early plant growth promoters under stressful environment.

Methods

This study used plant micribiome's approaches to advance the concept of plant prenatal care towards enhanced seed vigor/germination and crop production under stressful conditions.

Conclusions

Screening microbiome and testing microbial endophytes for plant growth promotion and resistance over the entire lifecycle may depict a *continuum* of the symbiosis benefits

FEMS7-1468
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

HYPER-ARSENIC RESISTANCE STRATEGY OF A BACTERIA: ORGANIZATION, FUNCTION AND EVOLUTION OF ARS GENES

L. Wang[1], C. Jing[1]
[1]Research Center for Eco−Environmental Sciences-Chinese Academy of Sciences, State Key Laboratory of Environmental Chemistry and Ecotoxicology, Beijing, China

Backgrounds

Arsenic (As) is a toxic element present in many environmental biotopes. To resist As disruptive effects, microbes have evolved a variety of mechanisms. IMH is the only bacterium found in genus *Pantoea* with a high As resistant capacity, but its molecular mechanism is unknown.

Objectives

The objectives of this study are 1) what genes are involved in the As resistance of IMH, 2) how As resistance genes work in the presence of As, and 3) how these genes are obtained.

Methods

Genome sequencing, genome annotation and analysis were used to find As resistance genes. Deleted mutants, heterologous expression, RT-Q-PCR analysis were performed to explore the function of As resistance genes. Phylogenetic analysis and the deviant G+C content were used to detect the evolution of As resistance genes.

Conclusions

Two *ars* systems - *ars1* (*arsR1B1C1H1*) and *ars2* (*arsR2B2C2H2*) - with low sequence homology and two *arsC*-like genes were found in the IMH genome. Both *ars1* and *ars2* are involved in the As resistance, where *ars1* is the major contributor at 15 ^{o}C and *ars2* at 30 ^{o}C. The contrasting performances of these two *ars* systems is attributed to the disparate activities of their *arsR* promoters under different temperatures. Sequence analysis based on concatenated ArsRBC indicates that *ars1* and *ars2* clusters may be acquired from *Franconibacter helveticus* LMG23732 and *Serratia marcescen*s (plasmid R478), respectively, by horizontal gene transfer (HGT).

FEMS7-2365
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

METATRANSCRIPTOME SEQUENCING REVEALS SHIFTS IN THE BOVINE EPIMURAL BACTERIAL COMMUNITY COMPOSITION WHICH ARE COMPENSATED ON A FUNCTIONAL LEVEL

S.U. Wetzels[1,2,3], *E. Mann*[1,2], *Q. Zebeli*[2,3], *M. Wagner*[1,2], *S. Schmitz-Esser*[2,4]
[1]*Institute for Milk Hygiene- Milk Technology and Food Science,*
Department for Farm Animals and Veterinary Public Health- University of Veterinary Medicine Vienna, Vienna, Austria
[2]*Research Cluster 'Animal Gut Health', University of Veterinary Medicine Vienna, Vienna, Austria*
[3]*Institute of Animal Nutrition and Functional Plant Compounds,*
Department for Farm Animals and Veterinary Public Health- University of Veterinary Medicine Vienna, Vienna, Austria
[4]*Department of Animal Science, Iowa State University, Ames, USA*

Backgrounds

The exploration of the bovine epimural bacterial microbiota (BEBM) in the rumen is relevant for basic and applied research into explaining the effect of subacute ruminal acidosis (SARA) on BEBM community structure and host-microbe interactions. Comparatively little is known about the metabolic function of the BEBM and whether or how it might be affected by a diet-induced SARA challenge.

Objectives

This study aimed to investigate differences in the gene expression of the BEBM before and after a diet-induced SARA challenge.

Methods

Three ruminally cannulated Holstein dairy cows were fed forage only during the baseline period followed by a five-week SARA challenge induced by a 60% concentrate diet. Cows were slowly adapted to the high-concentrate diet within the first 6 challenge days. Rumen papillae biopsies were taken at the baseline period and after five weeks SARA challenge. The metatranscriptome of the BEBM at the baseline period and after five weeks SARA challenge was obtained using Illumina HiSeq RNA sequencing.

Conclusions

In total, 291,664,242 sequences were obtained, assigned to 25 phyla, with *Proteobacteria*, *Firmicutes* and *Bacteroidetes* being the most abundant phyla. Functional assignments revealed carbohydrate and amino acid metabolism as well as translation to be the most transcribed KEGG/KO pathways. PCoA revealed clustering of BEBM community structure, which was not reflected in the functional data. We conclude that a diet-induced SARA challenge largely affects the composition of the BEBM without strong effects on the functional metatranscriptome, indicating that shifts in bacterial community composition are compensated on a functional level.

FEMS7-1299
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE CONSTRUCTION OF THE CURATED PRETERM GUT MICROBIOME DATABASE (PGMD)

K. Wong[1], P. Fleming[1], M. Millar[2], R. Hutchinson[1], M. Wilks[2], F. Stacey[3], K. Costeloe[1], W. Wade[4]
[1]Blizard Institute Queen Mary University of London, Centre for Genomics and Child Health, London, United Kingdom
[2]Barts and the London NHS Trust, Department of Infection, London, United Kingdom
[3]Homerton University Hospital NHS Foundation Trust, Neonatal Intensive Care Unit, London, United Kingdom
[4]Blizard Institute Queen Mary University of London, Centre for Immunobiology, London, United Kingdom

Backgrounds

The global survival rate for very low birthweight preterm babies has significantly increased over the past 30 years. Morbidity and mortality have shifted from early respiratory failure to intermediate complications such as late-onset sepsis and necrotising enterocolitis (NEC). Investigations into the pathophysiology underpinning NEC are centred on interactions between the preterm infant gut and its microbiome, with recent studies typically using culture-independent techniques such as 16S rRNA gene community profiling and next-generation sequencing to characterise the preterm gut microbiome.

Objectives

Currently available DNA sequence nucleotide databases are typically very large and often include a significant proportion of erroneous or poor-quality 16S rRNA gene sequences. The aim of this study was to construct a 16S rRNA gene Preterm Gut Microbiome Database (PGMD) that is both curated and site-specific, thus providing a robust resource for future preterm gut microbiome studies.

Methods

High-quality full-length 16S rRNA gene sequences representing all bacterial species reported in the literature for the preterm infant gut were compiled. Data from an experimental community profile dataset was used to interrogate the database. Where sequences showed ≥98.5% similarity to named species not in the database, those species were added. Un-named phylotypes represented by a high-quality reference sequence were also added. All species-level taxa were assigned a unique PGMD number.

Conclusions

The first iteration of the PGMD is comprised of 623 species-level taxa from the phyla *Acidobacteria*, *Actinobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Fusobacteria*, *Proteobacteria* and *Tenericutes*. The PGMD is a source of phylogeny-designated taxa useful for investigators involved in understanding the human microbiome.

FEMS7-1268
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

THE EFFECT OF ENVIRONMENTAL AND SEDIMENT CHARACTERISTICS ON THE SPATIAL AND TEMPORAL DISTRIBUTION OF E. COLI IN INTERTIDAL SEDIMENTS

A. Wyness[1,2], *D. Paterson*[2], *E. Defew*[2], *M. Stutter*[1], *L. Avery*[1]
[1]*The James Hutton Institute, Environmental and Biochemical Sciences, Aberdeen, United Kingdom*
[2]*University of St Andrews, School of Biology- Scottish Oceans Institute, St Andrews, United Kingdom*

Backgrounds

Current bathing water quality monitoring, in accordance with microbial water quality regulations, ignores the presence of faecal indicator organisms (FIOs) in river or estuarine sediments. Sediments act as an environmental reservoir of FIOs, therefore when sediments are resuspended, FIOs are redistributed into the water column and can contribute significantly to adverse microbial water quality. Understanding FIO distribution and abundance in sediments therefore provides an improved understanding of microbial pollution and increase the accuracy of bathing water quality advisories.

Objectives

The aim of this work was to identify and model key driving factors that influence the spatial and temporal variation in the abundance of *E. coli* in intertidal estuarine sediments.

Methods

Intertidal sediments were collected from two estuaries in NE Scotland through seasonal transect sampling, and a spatially and temporally intensive regime. Enumeration of *E. coli* from surface sediments was performed in conjunction with a comprehensive suite of physical, biogeochemical and biological sediment analyses. Data were explored using Spearman's rank correlation coefficients, best subsets models and multiple stepwise linear regression models.

Conclusions

E. coli abundance was largely dependent on biogeochemical variables, with interstitial pore water salinity consistently influential. Multiple linear regression models of the different datasets explained up to 87.4 % of *E. coli* variation suggesting that, with further research, the predictive modelling of FIO abundance in sediments could be included in the designation of bathing water quality advisory notices.

FEMS7-1664
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ARCHAEAL COMMUNITIES ALONG A 2M LONG SEDIMENT CORE IN THE DAJIUHU PEATLAND, CENTRAL CHINA

X. Xiang[1], R. Wang[1], Y. Xu[1], W. Tian[1], L. Gong[2], H. Wang[1]
[1]China University of Geosciences- Wuhan,
State Key Laboratory of Biogeology and Environmental Geology, Wuhan, China
[2]Third Institute of Oceanography, Third Institute of Oceanography, Xiamen, China

Backgrounds

Archaea has been recognized as important motor driving various biogeochemical processes in natural environments, while knowledge regarding archaeal community structure and their relationship with environment factors is limited particyularly in peatland ecosystems

Objectives

the vertical distribution of archaeal communities along a 2 m peat sedimentary core from the Dajiuhu Peatland

Methods

quantitative PCR and next sequencing methods

Conclusions

Quantitative analysis showed that archaeal abundance ranged from 10^5 to 10^7 copies per gram sediment, highest at 1 m depth of peat sediments. Taxonomically archaeal communities consisted of 8 archaeal phyla and were predominated by Miscellaneous Crenarchaeota Group (MCG) with a relative abundance of 90%, followed by Thaumarchaeota and Crenarchaeota. Furthermore, members of MCG were split into 9 subgroups suggesting a highly abundant and diverse MCG lineage in peat sediment. Water content and TOC were proved to be the most significant factors to architecture archaeal community structures as indicated by both phylogenetic and taxon-based approaches. Network analysis revealed that MCG strongly control other archaeal lineages through interaction and may play important role in biogeochemical processes in peatland ecosystem. Our results provided overview of archaeal population, community structure, relationship with environmental factors and emphasized the potential ecological function of MCG lineages

FEMS7-0456
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MECHANISM OF RADIATION RESISTANCE IN ARTHROSPIRA SP. PCC 8005

A. Yadav[1], N. Leys[1], A. Cuypers[2], A. Misztak[3], K. Waleron[3], M. Waleron[4], P. Janssen[1]
[1]SCK-CEN, Molecular and cellular Biology, Antwerpen, Belgium
[2]University Of Hasselt, Centre for Environmental Sciences, Hasselt, Belgium
[3]University of Gdańsk, Department of Biotechnology, Gdańsk, Poland
[4]Medical University of Gdańsk, Pharmaceutical Microbiology, Gdańsk, Poland

Backgrounds

The cyanobacterium *Arthrospira* is globally used as feed- and foodstock owing to its high nutritional value. We study *Arthrospira* sp. strain PCC 8005 as a principal organism and edible end product of MELiSSA, a life support system developed by ESA. Particular caveats to study this highly versatile organism are its assay-interfering auto-fluorescence and the lack of a genetic system.

Objectives

Two important aspects of our research are (i) to understand the genetic and biochemical pathways involved in the resistance of strain PCC 8005 to high doses of ionizing radiation (IR) and (ii) to define optimal growth conditions for *Arthrospira* to conserve its beneficial properties in radiation-intensive space environments.

Methods

To study the effect of radiation on growth and morphology and to test whether extreme IR-resistance is a general trait in *Arthrospira*, different strains were exposed to increasing doses of gamma radiation (up to 5 kGy) and analyzed for culture-based growth recovery, morphological changes, and cellular and molecular effects (by TEM microscopy and LC-MS). Strain PCC 8005 was also exposed to IR during at least one full cycle of LED-driven photosynthetic growth for RNAseq analysis.

Conclusions

Arthrospira strains and species from different origins display a variable sensitivity towards IR. Even the two morphotypes of *Arthrospira* sp. strain PCC 8005 (straight versus helical) show, in terms of growth recovery, distinct sensitivities towards IR. Once all data are analyzed, LC-MS, TEM, and RNAseq analyses should provide a detailed insight in the cellular responses of *Arthrospira* sp. PCC 8005 towards IR.

FEMS7-2936
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

GENOME MINING OF NOVEL BACTERIAL STRAINS FOR POTENTIAL CRISPR/CAS SYSTEMS

S. Yadav[1], J. Hurr K[2], S.S. Lee[1]
[1]Kyonggi University, Life Science, Suwon, Republic of Korea
[2]Korea University, Stem Cell Institute-, Seoul, Republic of Korea

Backgrounds

Clustered regularly interspaced short palindromic repeats (CRISPR) are segments of prokaryotic DNA containing short, repetitive base sequences. Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA. It is an adaptive immune system of prokaryotes which employ short sequences, called spacers, which guide Cas proteins to cleave foreign DNA. The CRISPR/Cas systems are present in majority of the archaeal and about 40% of the bacterial genome. However, there are very limited numbers of bacteria are explored for the novel CRISPR/cas system. Hence, exploration of previously uncultivated bacterial strains could be the potential target for the novel CRISPR/cas systems.

Objectives

This study was designed to explore the genomes of novel bacterial strains which could be potential target for the novel CRISPR/cas systems. A total of 100 novel bacterial species collected from Korean Environmental Microorganisms Bank (KEMB), Kyonggi University, South Korea.

Methods

Genomes of the all the bacterial strains were sequenced and analysed for the presence of novel CRISPR/cas system. Based on preliminary analysis a total of about 31 bacterial genomes belong to the phyla *Proteobacteria*, *Spirochaetes*, *Planctomycetes* and *Fusobacteria* were found to possess the CRISPR/cas system. Out of these strains only *Enhydrobacter* sp. strain H5 were found to possess a new sub-type of the CRISPR/cas system while rest bacteria contained previously known CRISPR/cas systems.

Conclusions

Genome and transcriptome analysis of *Enhydrobacter* sp. strain H5 confirmed that this strain possess a novel sub-type of CRISPR/cas 9 system.

FEMS7-2209
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

MICROBIAL DIVERSITY AND ICE NUCLEATION PROPERTIES OF BIOAEROSOL SAMPLES COLLECTED IN THE EASTERN MEDITERRANEAN

P. Yordanova[1], S. Maier[1], N. Lang-Yona[1], A. Tamm-[1], H. Meusel[1], U. Pöschl[1], J. Fröhlich[1], B. Weber[1]
[1]Max Planck Institute for Chemistry, Multiphase chemistry, Mainz, Germany

Backgrounds

Primary biological aerosols (bioaerosols), a subset of atmospheric particles, comprise living and dead microorganisms, reproduction units and organism fragments. Bioaerosols can act as cloud condensation and ice nuclei, therefore they have potential to modify cloud albedo, precipitation, and the hydrological cycle.

Objectives

The present study aims at accomplishing two major tasks: 1) identification and quantification of bioaerosol particles and 2) investigation of ice nucleating (IN) ability of total suspended particles (TSP) collected at the remote site Agia Marina Xyliatou in Cyprus during a field campaign in April 2016.

Methods

Total suspended particles of various types of air masses such as African (Saharan) and Arabian dust and European and Middle Eastern pollution were collected on glass fiber filters at 24 h intervals. During the sampling period, two major dust storms (PM 10_{max} 119 µg/m^3 and 64 µg/m^3) and a rain event were documented.

The samples are investigated for microbial composition by metagenomics and IN properties are characterized by filtration, thermal, chemical and enzyme treatments. Immersion freezing experiments are performed at relatively high subzero temperatures (-1 to -15°C) using a mono ice nucleation assay.**Conclusions**

Preliminary results indicate that highest IN particle numbers (INPs) occurred during the second dust storm event characterized by lower particle concentrations. Treatments at 60°C lead to gradual INP deactivation, indicating the presence of biological INPs. Additionally, first results indicate that bacteria dominate the microbial community with Firmicutes, Actinobacteria, Proteobacteria and Cyanobacteria being the most abundant phyla.

FEMS7-2140
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

EFFECTIVE BIODEGRADATION OF HEAVY METAL FREE AND POLLUTED FEATHER WASTES BY GELLAN GUM IMMOBILISED BACILLUS SP.

I. Yusuf[1], *S. Yahaya*[1]
[1]*Bayero University Kano- Nigeria, Microbiology, Kano, Nigeria*

Backgrounds

Waste waters from residential areas in many cities in Nigeria are not effectively discharged and constitute part of environmental pollution by forming sludge that harbor many bacteria and parasites.

Objectives

With a view to utilising cells of heavy metal tolerant bacterium- *Bacillus sp.* isolated from domestic waste sludge in degradation of polluted feather wastes, an investigation was made of the ability of gellan gum immobilised cells of the bacterium to degrade heavy metal polluted feathers and produce keratinase.

Methods

The performance of the immobilized cells in the degradation of heavy metal free and polluted feather wastes was evaluated. Compared with the free cells, the immobilised cells was able to tolerate broader range of heavy metal concentration, produced higher keratinase and degrade heavy metal polluted feathers faster. After repeated use, beads of immobilised Bacillus sp retained about 90% of its feather degrading ability (FDA) without need for desorption for 7 consecutive cycles. Although FDA decreased considerably when concentration of Hg, Pb, Mn and As, Cd, is above 5 and 10 ppm respectively, but it maintained about 76% of FDA during repeated degradation of feathers polluted with 5 and 10 ppm respectively. When mixture of Ag, Co, Cu at 25 ppm each were used, FDA decreased to 55 and 32% at the end of 4 and 6 cycles respectively.

Conclusions

The study indicates that, the use of immobilised cells of Bacillus sp offers a promising means of bioremediation of heavy metal polluted feather wastes and in the production of keratinase enzymes.

FEMS7-0066
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ISOLATION OF SULFUR OXIDIZING BACTERIA FROM NAMAKDAN CAVE IN QESHM

N. Zamani[1], M.A. Amozegar[1], M. Mehrshad[2]
[1]Faculty of Biology - College of Science - University of Tehran -, microbiology, Tehran, Iran
[2]institute of hydrobiology, Aquatic microbial ecology, Ceske Budejovice, Czech Republic

Backgrounds

Organisms require energy for life processes such as growth and movement. Those bacteria which oxidize inorganic compounds with molecular Oxygen to acquire energy, are called chemolithotrophic bacteria. They can oxidize Ammonia , Sulfur and reduced sulfur compounds , nitrite , ferrus iron , hydrogen and etc . These kinds of bacteria were discovered by S.N.Winogradsky in 1891 for the first time

Objectives

This study aims at isolating Sulfur Oxidizing chemolithotrophic Bacteria from salt cave Qeshm. Based on the latest findings of geologists, this cave is considered as the longest salt cave in the world. and it is located in the southern coast of Iran (Persian Gulf).

Methods

After primary sampling considering the existing compounds in the natural environment of this cave, the researcher in this study has adapted the amounts of compounds in the culture medium and has been prepared in three NaCl concentrations of 0.2, 2, and4 M. Following the main sampling, the samples were taken to the laboratory and they were inoculated at enrichment medium in 250cc erlen. They were placed into the incubator shaker in a tempreture of 30 ° C and 150 rpm for 8 weeks. The act of second and third subculturing were conducted in 8 weeks intervals. The third subculture were transfered into the solid medium and three steps of purification were carried out and sixteen pure isolates were obtained

Conclusions

Amongst these strains we chose strains SN1 , SN2 , SN3 , SN4 for further characterization and each of these strains showed 16S rRNA gene sequence similarity with *Halothiobacillus hydrothermalis* RT3(98.83% , 99.2 %,98.91% , 98.62 %) respectively.Based on some morphological tests: Cells were gram negative Bacills with different features in catalase and oxidase tests. This study is the first step in characterization of chemolithotrophic bacteria and their role in saline cave ecosystems.

FEMS7-2872
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ANTIMICROBIAL ACTIVITY OF PHARMACEUTICALS WITH A CARBAZOLE RING IN THEIR STRUCTURE

K. Zawadzka[1], A. Nejman[1], K. Lisowska[1]
[1]Univesity of Lodz, Department of Industrial Microbiology and Biotechnology, Lodz, Poland

Backgrounds

The mammals stay in close association with their microbiome including archea, bacteria and fungi settle down in their organisms. The microbiome of living organisms is extremly diverse and striking a correct balance of microflora is a key factor in maintaining the positive state of health. In recent years, human and animal microbiomes have been shown to be affected by various drugs especially antibiotics. Carbazole is an N-heterocyclic compound commonly used in the production of dyes and drugs. Carvedilol and ondansetron have a carbazole ring in their structure. Carprofen is a non-steroidal anti-inflammatory drug (NSAID) of the propionic acid class. Ondansetron is a serotonin 5-HT3 receptor antagonist used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy and surgery.

Objectives

The aims of this study were to evaluate antimicrobial activity of carprofen and ondansetron against Gram-positive and Gram-negative bacteria and to assess its impact on the mammalian microbiome.

Methods

Antimicrobial activity of the tested drugs was examined by the modified method of serial dilutions in Miller-Hinton medium in accordance with the standard by The European Committee on Antimicrobial Susceptibility Testing involving spectrophotometric measurement of the optical density of microbial growth with serial dilutions of carprofen or ondansetron.

Conclusions

The influence of carprofen and ondansetron on the growth of both Gram-positive and Gram-negative bacteria was confirmed. The obtained results indicate that these drugs may interfere with the microbiome balance by inhibiting and simulating of the growth of different microorganisms.

FEMS7-3100
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

INVESTIGATION ON THE DIFFERENTIATION OF TWO USTILAGO ESCULENTA STRAINS - IMPLICATIONS OF A RELATIONSHIP WITH THE HOST PHENOTYPES APPEARED IN THE FIELDS

Y. Zhang[1], *Z. Ye*[1]
[1]*China Jiliang University,*
Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, Hangzhou, China

Backgrounds

Ustilago esculenta, a pathogenic basidiomycete fungus, infects *Zizania latifolia* to form edible galls named jiaobai in China. Distinct growth conditions of *U. esculenta* resulted *Z. latifolia* to form three different phenotypes, named male Jiaobai, grey Jiaobai and white Jiaobai respectively.

Objectives

The aim of this study was to elucidate the differentiations of the two *U. esculenta* strains (named T and MT) isolated from grey Jiaobai and white Jiaobai respectively.

Methods

The different infection ability and growth response to environment cues, like pH, distinct nitrogen or carbon sources, between T and MT strains, were verified by morphological observation with microscope and inoculation assay. Meanwhile, the non-synonymous mutation analysis based on the comparative genome sequencing database between the MT and T strains was carried out to be further evidence.

Conclusions

MT strains appeared slower growth rate and fewer growth mass both in sporidia and hyphae, more sensitive to external signals, including pH, oxidative stress and nutritions except arginine. Also, the non-synonymous mutation analysis showed multiple mutations in pathogenic genes of MT strains, in accordance with their reduced pathogenicity when compaired to T-type strains. So it was reasonable to speculate the relationship between *U. esculenta* strains differentiations and their host phenotypes.

FEMS7-0050
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PHOSPHATE MINERAL DIFFERENTIATED BACTERIAL AND FUNGAL COMMUNITIES FROM WEATHERED ROCKS IN HESHANG CAVE

J. Zhou[1]
[1]*China University of Geosciences, School of Environmental Studies, Wuhan, China*

Backgrounds

As a unique habitat, rock surface harbor various microbial communities which extract their nutrients from the rocks via their metabolic activities. However knowledge about microbial communities on the surface of cave wall rocks and the environmental factors controlling their distribution are still lacking.

Objectives

This study was designed to investigate bacterial and fungal communities and their correlation with mineral compositions in Karst Cave wall rocks.

Methods

We collected rock samples according to the different zones and different layers on the wall in Heshang Cave, central China. Bacterial and fungal diversity from rocks surfaces, crust layers and loose layers, as well as microbes in photic zone, twilight zone and aphotic zone were examined via 16S rRNA and ITS Illumina amplicon sequencing. Meanwhile mineral composition of samples were analyzed by X-ray diffraction.

Conclusions

In total 4,969 bacterial OTU and 989 fungal OTUs were recovered with an identity of 97 %. Both bacterial and fungal compositions showed significant differences among samples from photic zone and other two zones at the phylum lever and the class level respectively. Actinobacteria and Sordariomycetes dominate bacterial and fungal communities in most samples. RDA analysis indicated that hydroxylapatite significantly impacts bacterial and fungal communities, while fungal communities also showed a close correlation with whitlockite. Together, these results suggest that cave weathered rocks harbor a high diversity of bacterial and fungal communities, which are closely related to phosphate mineral.

FEMS7-0252
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

HOW TO TRANSMIT A GUT BACTERIAL SYMBIONT IN SOCIAL INSECTS?

M. Zhukova[1], *P. Sapountzis*[1], *M. Schiøtt*[1], *J.J. Boomsma*[1]
[1]*University of Copenhagen, Centre for Social Evolution- Department of Biology, Copenhagen, Denmark*

Backgrounds

The transmission of bacterial symbionts across host generations is crucial for the direction and efficiency of host-symbiont coevolution. Specialized gut microbes of ants are peculiar because within-colony horizontal transmission can produce exclusive vertical transmission across generations. Colony life may thus alleviate constraints in the pupal stage where direct transmission from larval- to adult guts is impossible, particularly when the pupal environment is predictable.

Objectives

We analysed gut microbiome changes across developmental stages and traced the bacterial transmission routes of two abundant gut symbionts of *Acromyrmex* and *Atta* leafcutter ants, which are important functional herbivores in the New World tropics.

Methods

We analysed Illumina MiSeq 16S rRNA gene amplicon sequencing data to determine gut bacterial communities at different developmental stages. We also measured bacterial abundance using droplet digital PCR, and used confocal and electron microscopy to visualize bacteria in different gut tissues.

Conclusions

The larval and pupal guts contained several bacterial species that also occur in the fungus gardens, while adult workers hardly have these bacteria in spite of eating the same fungal material. Non-parasitic *Wolbachia* were most abundant across developmental stages in *Acromyrmex*, but *Atta* had mostly Mollicutes in the worker guts. *Wolbachia* appears to be transovarially transmitted, but Mollicutes were absent in eggs and scarce/absent in larval and pupal guts. After eclosion, callows acquired Mollicutes through interactions with other workers and failed to obtain these bacteria in isolation, suggesting that Mollicutes are socially transmitted. Both pathways result in exclusive vertical transmission across generations and should therefore facilitate host-symbiont coevolution in similar ways.

FEMS7-0746
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

ENVIRONMENTAL ORDINATION OF FILAMENTOUS BACTERIA IN ACTIVATED SLUDGE

A. Zornoza[1], *S. Serrano*[2], *J.L. Alonso*[1]
[1]*Ingeniería del Agua y Medio Ambiente, Área de Química y Microbiología del Agua, Valencia, Spain*
[2]*Universidad Complutense de Madrid. Facultad de Biología, Departamento de Microbiología III, Madrid, Spain*

Backgrounds

Studies about the dynamics of filamentous bacterial populations in activated sludge, have often been on bulking and foaming episodes, mainly focused to clarify the taxonomic position, in situ ecophysiology, presence and distribution, as well as the strategies for controlling the populations. However, the studies about the filamentous bacteria from the point of view of the environmental interpretation are still scarce.

Objectives

A relationship between filamentous bacteria and environmental variables was carried out. This study have been focused on determining the ordination of the relationships between filamentous bacteria and physicochemical, and operational variables in full-scale wastewater treatment plants (WWTPs) operating in temperate climate.

Methods

Samples from activated sludge, influent and treated effluent were collected fortnightly during a year from six bioreactors belonging to four different WWTPs located in Spain. In our study we combined the conventional microscopy and the fluorescent in situ hybridization (FISH) using 27 FISH probes for filamentous bacteria to estimate the absolute density of filamentous bacteria in the bioreactors. Distance-based linear models (DISTLM) were applied to investigate models of environmental interpretation of biological variables.

Conclusions

The construction of models allows associating the filamentous bacteria to environmental ranges, obtaining valuable information to the knowledge of these dynamic populations. Our results provide insights into dynamics of filamentous bacteria in response to physicochemical and operational parameters changes in activated sludge systems and improve our understanding regarding the effect of these variables on wastewater treatment efficiency.

FEMS7-0750
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

PLAUSIBLE BIOINDICATORS OF BIOLOGICAL NITROGEN REMOVAL PROCESS IN WWTPS. APPLICATION OF MULTIVARIATE PREDICTED MODELS

A. Zornoza[1], *J.L. Alonso*[1], *S. Serrano*[2]
[1]*Ingeniería del Agua y Medio Ambiente, Área de Química y Microbiología del Agua, Valencia, Spain*
[2]*Universidad Complutense de Madrid. Facultad de Biología, Departamento de Microbiología III, Madrid, Spain*

Backgrounds

The optimization of wastewater treatment involves the search of new tools for the control of the process. Nowadays, restrictions concerning the discharge of certain pollutants, such as nitrogen and phosphorus are imposed, so new configuration of treatment plants have been proposed. Advances in the knowledge of dynamics of the protists and metazoans populations are necessary for the proposal of new biological control tools in activated sludge. Many studies have attempted to relate protists and metazoans with physicochemical and operational variables of wastewater treatment plants (WWTPs) in order to reveal possible bioindicators. However, these studies have been mainly descriptive and/or exploratory and environmental interpretation has not been included in them.

Objectives

The aim of this work is the proposal of biological bioindicators of the nitrogen removal process.

Methods

Samples from activated sludge, influent and treated effluent were collected fortnightly during a year, from six bioreactors belonging to four different WWTPs (Spain). Density of protists and metazoans was obteined by direct counting and different staining procedures, using phase contrast microscopy. Models of environmental interpretation of biological variables, distance-based linear models (DISTLM), as well as the canonical correspondence analysis (CCA), were applied.

Conclusions

The multivariate models constructed with DISTLM and CCA have provided relevant information about the relationships between protists and metazoans and some plant nitrogen compounds, allowing their ecological interpretation and obtaining new bioindicators for monitoring the biological nitrogen removal process in active sludge.

FEMS7-1417
Environmental Microbiology/Microbial Ecology /Microbial Communities - Part III

BACTERIAL POPULATIONS IN NATURAL GAS SEEPAGE POCKMARKS AND ASSOCIATED BIOTOPES IN NORTH-EAST ICELAND

G.V. Thorsteinsdottir[1], A. Blischke[2], T.S. Arnarson[3], O. Vilhelmsson[1]
[1]University of Akureyri, Faculty of Natural Resource Science, Akureyri, Iceland
[2]Iceland Geosurvey, Branch Akureyri, Akureyri, Iceland
[3]Orkustofnun, Hydrocarbon Exploration, Reykjavík, Iceland

Backgrounds

Natural gas seepage pockmarks present ideal environments for bioprospecting for alkane and aromatic degraders, and investigation of microbial populations with potentially unique adaptations to the presence of hydrocarbons.

Objectives

On-shore seepage pockmarks are found at two disparate sites in the Jökulsá á Fjöllum delta in North-East Iceland.

Methods

The origin and composition of headspace gas samples from the pockmarks were analysed by GC-MS and stable isotope analysis, revealing a mixture of thermogenic and biogenic gases with considerable inter-site variability. The warmer, geothermally impacted site displayed a more thermogenic character, comprising mostly methane and CO2 with minor amounts of higher alkanes. The water chemistry of the pockmark sites was determined, revealing considerable heterogeneity between sites. The geothermally impacted site water contained higher amounts of calcium and zink, and lower amounts of iron than the more biologically impacted site. Microbial communities were analysed by 16S rDNA tag sequencing of extracted DNA from the gas seepage pockmarks and additional sediment samples were taken for culturing and isolating bacterial strains on selective media.

Conclusions

The bacterial community of the thermogenic gas site is mostly composed of the phyla *Chloroflexi* (38%), *Proteobacteria* (8%) and *Firmicutes* (4%). *Dehalococcoidia* were by far the most abundant class, indicating a community strongly affected by anaerobic dehalogenation. The biogenic gas site showed higher microbial diversity, dominated by the phyla *Proteobacteria* (31%), *Bacteriodetes* (14%) and *Chloroflexi* (10%). Studies on isolated strains have revealed degraders of naphthalene, hexane and propane, as well as potentially facultative dehalorespirers, and bacteria likely to display bioweathering and biodeposition activities.

FEMS7-1267
Food Microbiology

CHARACTERIZATION OF THE MOLECULAR DETERMINANTS INVOLVED IN THE COLONISATION ABILITY OF ESCHERICHIA COLI O157:H7 TO THE EXTRACELLULAR MATRIX

V. AGEORGES[1], *N. CACCIA*[1], *C. CHAGNOT*[1], *C. PROVOT*[2], *F. CHAUCHEYRAS-DURAND*[1], *M. DESVAUX*[1]
[1]*French National Institute for Agricultural Research, MICA, Saint-Genès Champanelle, France*
[2]*Biofilm Control, Biofilm Control, Saint-Beauzire, France*

Backgrounds

Among intestinal pathogenic *Escherichia coli* (InPEC), enterohaemorrhagic *E. coli* (EHEC) 0157:H7 are anthropozoonotic agents leading to haemorrhagic colitis and haemolytic-uremic syndrome (HUS). From ruminants (the animal reservoir), EHEC can contaminate some foodstuffs and consequently infect human. While the locus of enterocyte effacement (LEE) is generally considered as a key determinant of EHEC, it is neither sufficient nor systematically present in all EHEC strains. The presence of extracellular matrix (ECM) components along the food chain could increase their colonisation capacity. Numerous proteins can be present at the bacterial cell surface of EHEC and could participate to the bacterial adhesion and biofilm formation at the ECM.

Objectives

This study aimed at identifying and characterizing the respective involvement of cell-surface proteins in the colonisation process of EHEC to some ECM components.

Methods

Target genes were identified by a proteogenomic approach based on the secretome concept. The involvement of these genes in bacterial adhesion and biofilm formation was characterized following functional genetic analysis.

Conclusions

Several organelles and adhesins, either secreted by the Type I secretion system (T1SS) to the Type VIII (T8SS), could identified in EHEC and related enteropathotypes. Following gene knock-out, the ability of the mutants to adhere and/or to form biofilm to ECM components was compared to the wild type strain. Besides the flagella (T3bSS) and pili of the T7SS, some autotransporters belonging the T5aSS appeared to contribute significantly to specific and/or non-specific colonisation of the ECM by *E. coli* O157:H7.

FEMS7-2755
Food Microbiology

RISK ASSESSMENT OF FEED ADDITIVES OF MICROBIAL ORIGIN IN THE EUROPEAN UNION

J. Aguilera[1], *R. Brozzi*[1], *M. Anguita*[1], *J. Galobart*[1]
[1]*European Food Safety Authority- EFSA, FEED Unit, Parma, Italy*

Backgrounds

Microorganisms can be used in animal nutrition as probiotics, silage agents or as a source of other additives like enzymes, amino acids and vitamins. In the European Union, Regulation (EC) No 1831/2003 stipulates that all feed additives intended to be marketed need to undergo a risk assessment, which is conducted by the European Food Safety Authority.

Objectives

A proper characterisation of the microorganism is fundamental for its safety assessment. This includes non-equivocal identification of the species, consideration of its pathogenic or toxigenic potential and its resistance to antimicrobials. Genetically modified strains require a full characterisation of the modification.

Methods

The characterisation of the microorganism determines the need of further tests to be done in the product to address the safety aspects related to the production strain. For example, if the strain is capable of producing toxic compounds, the product should be free of those compounds. Toxicological tests may be necessary if the safety of the strain cannot be established. Conversely, if the production microorganism has a well-documented safety, it may be eligible for a Qualified Presumption of Safety status, in which case it is considered safe without the need of further testing.

Conclusions

Within the activity of updating its guidance documents for the risk assessment of feed additives, EFSA will issue a unique guidance document covering all data needed for the characterisation of microorganisms, both as products or as a source of them. The document aims to streamline the process of preparation and assessment of dossiers for microbial and microbial-based additives.

FEMS7-0051
Food Microbiology

EFFECT OF REPLACING ANTIBIOTIC (AGPS) WITH POLYPHENOLIC BOTANICAL EXTRACTS AND ESSENTIAL OILS IN FEED OF LAYING HENS ON PERFORMANCE, HEALTH, ND TITER AND BLOOD PARAMETERS

A. Ahmad[1]
[1]*Karachi, Pakistan*

Backgrounds

Polyphenolic vegetative extracts and essential oils are very important replacement additives for the antibiotic growth promoters in layer feeds. The goal of this study was to investigate the effect of vegetative extracts and essential oils on egg producing layers' health, immunity against ND virus disease and other blood parameters. One hundred and twelve, nine months old, white Novagin egg producing laying hens (1480+50 grams average live body weight each) were randomly distributed to seven groups equally (n = 16). Each group/treatment was divided four times with four birds per replicate cage unit. Diets (rations) were prepared by adding vegetative polyphenolic extracts of black tea (c), seeds of black cumin (d), fenugreek seed (e) as well as oils from black cumin seeds (f) and fenugreek seeds (g) in the negative control (b; treatment with nil antibiotic and antioxidant added in the ration) and compared with positive control (a) having antibiotic (4.4%) lincomysin 120mg/kg of feed, acetic acid (99.5%) 0.15mL/kg of feed, antioxidant seldox (BHA, BHT, ethoxiquine and citric acid) 120mg/kg of feed. After end of the five weeks trial, weekly feed conversion ratio (FCR) of all treatments were significantly good than negative control (b) ($P<0.05$), however treatments a, b, d, e, f and g had non-significant differences among one another ($P>0.05$). ND titer level remain same of all treatments and overall health performance was good and there was insignificant difference between all blood parameters except negative control ($P>0.05$). These results clarify that polyphenolic vegetative extracts/oils have positive affect on the performance, health and there is no drastic change in blood parameters of the egg producing layers and thus can be replaced by antibiotic growth promoters.

Objectives

Observation of polyphenolic vegetative extracts effect on birds heath and performance.

Methods

Used to see effects by feeding birds

Conclusions

We can replace antibiotics with vegetative extracts.

FEMS7-1851
Food Microbiology

ANTIBACTERIAL ACTIVITY OF CHILEAN ULMO (EUCRYPHIA CORDIFOLIA CAV.) HONEY AGAINST WOUNDS AND FOOD PATHOGENS

C. Burgos-Díaz[1], M. Farfán[2], F. Acevedo[3], V. Albarral[4]
[1]Agriaquaculture Nutritional Genomic Center- CGNA- Technology and Processes Unit, Technology and Processes Unit, Temuco, Chile
[2]Universitat de Barcelona, Facultat de Farmàcia i Ciències de l'Alimentació- Departament de Biologia Sanitat i Medi Ambient- Se cció Microbiologia, Barcelona, Spain
[3]Universidad de La Frontera, Scientific and Technological Bioresource Nucleus- BIOREN, Temuco, Chile
[4]Universitat de Barcelona, Facultat de Farmàcia i Ciències de l'Alimentació- Departament de Biologia Sanitat i Medi Ambient- S ecció Microbiologia, Barcelona, Spain

Backgrounds

Ulmo honey, derived from the Patagonian Eucryphicean tree (*Eucryphia cardifolia*) native to the rainforest of southern Chile, is considered the only honey in the world of botanical origin. It has been used to treat infections in traditional medicine in the Mapuche culture since ancient times.

Objectives

The main aim of this study was to analyze the antimicrobial activity of Ulmo honey against different pathogen species, including Gram-positive and Gram-negative bacteria and a yeast.

Methods

96-well Microtiter plates were used to determine the minimum inhibitory concentration (MIC). The assays were performed in quadruplicate. The selected bacterial strains comprised five wound pathogens, four food pathogens and one yeast, *Candida albicans.*

Conclusions

Ulmo honey exhibited strong antibacterial activity against *Klebsiella pneumoniae*, *Salmonella enterica* and *Staphylococcus aureus* MRSA (MIC 3.1%, v/v). In comparison with other honeys (e.g. Manuka honey), Ulmo honey presented a higher antimicrobial activity against *Staphylococcus aureus* MRSA, *Salmonella enterica* and *Klebsiella pneumoniae*, and similar activity against *Escherichia coli* and *Pseudomonas aeruginosa*. In contrast, *Bacillus subtilis*, *Enterococcus faecalis* and *Candida albicans* were unaffected, as in assays with other honeys. The results indicated that Ulmo honey displays strong inhibitory activity against pathogens usually isolated from wounds and ulcers and could be used as a potential therapeutic agent to treat bacteria resistant to drugs, such as *Staphylococcus aureus* MRSA, which is responsible for serious skin infections in humans.

FEMS7-2389
Food Microbiology

DEVELOPMENT OF REAL-TIME PCR ASSAYS FOR SEROTYPING LISTERIA MONOCYTOGENES IN MEAT PROCESSING PLANTS

A. Alía[1], A. Rodríguez[1], J.J. Córdoba[1], E. Bermúdez[1], P. Padilla[1], M.J. Andrade[1]
[1]University of Extremadura, Animal Production and Food Science, Caceres, Spain

Backgrounds

Listeria monocytogenes constitutes the main concern of Ready-To-Eat (RTE) meat products since its high risk for consumers, being the serotypes 1/2a, 1/2b, 1/2c and 4b the most frequently involved in human listeriosis. Given that this microorganism is a persistent contaminant in raw materials, equipment and utensils in meat industries, rapid and sensitive methods able to serotype *L. monocytogenes* are needed for characterizing its contamination source.

Objectives

The objective of this work was to develop sensitive SYBR Green-based real-time PCR (qPCR) assays to trace the source of contamination of *L. monocytogenes* in RTE meat processing plants.

Methods

To differentiate the four serotypes, the marker genes used were *lmo0737* (1/2a and 1/2c), *lmo1118* (1/2c), ORF2819 (1/2b and 4b) and ORF2110 (4b). The specificity and reliability of the qPCR methods were evaluated using 4 *L. monocytogenes* serotype reference strains and 20 isolates from meat industries.

Conclusions

The qPCR assays showed Ct values of 26.8 and 28.7 for *lmo0737* (1/2a and 1/2c, respectively), 24.9 for *lmo1118* (1/2c), 25.8 and 26.6 for ORF2819 (1/2b and 4b, respectively) and 19.2 for ORF2110 (4b). Nonspecific amplifications were observed. Results agreed with those obtained by a previously validated PCR method. The optimized qPCR protocols provide sensitive and rapid tools for serotyping *L. monocytogenes* isolates and could be very useful for characterizing sources contamination in RTE meat industries.

Acknowledgements: This work was funded by the projects RTA2013-00070-C03-03 of the INIA, GR15108 of the Government of Extremadura and FEDER. P. Padilla is recipient of a fellowship of Spanish Youth Guarantee Project (PEJ2014-P-0057).

FEMS7-2402
Food Microbiology

GENE EXPRESSION AS INDICATOR OF OCHRATOXIN A CONTAMINATION IN DRY-CURED FERMENTED SAUSAGES

V. Bernáldez[1], M.J. Andrade[1], L. Sánchez-Montero[1], A. Alía[1], M. Álvarez[1], J.J. Córdoba[1]
[1]University of Extremadura, Animal Production and Food Science, Caceres, Spain

Backgrounds

Ochratoxin A (OTA)-producing moulds such as *Penicillium nordicum* and *P. verrucosum* may colonise surface of dry-cured fermented sausages throughout their ripening process. This supposes a hazard of OTA accumulation with the subsequent risk for consumers. For this reason, it is necessary to have sensitive methods for detecting OTA-producing moulds before mycotoxin is produced, which may allow taking corrective actions during processing to avoid production of this mycotoxin. Gene expression analysis by reverse transcription real time PCR methods could allow detecting the presence of viable OTA-producing moulds. For this purpose the key genes involved in OTA biosynthesis could be tested.

Objectives

The objective of this work was to evaluate the temporal changes in OTA-related genes as indicator of phenotypic OTA production in dry-cured fermented sausages.

Methods

For this purpose slices of dry-cured fermented sausage "*salchichón*" were inoculated with *P. nordicum* and *P. verrucosum* and incubated up to 14 days at 97% relative humidity and 25ºC to favour mould growth. Temporal changes in the expression of *otapks* and *otapns* genes and OTA production were then evaluated.

Conclusions

Gene expression was higher in *P. nordicum* than in *P. verrucosum*. The high significant correlation found between the early relative expression of the *otapks* gene and OTA production lead to propose the *otapks* gene expression analysis as indicator of OTA accumulation in this dry-cured meat product.

Acknowledgements: This work was funded by Spanish Ministry of Economy and Competitiveness, Government of Extremadura and FEDER (AGL2013-45729-P, GR15108).

FEMS7-1141
Food Microbiology

EFFECT OF TEMPERATURE ON THE PREVALENCE OF SACCHAROMYCES NON CEREVISIAE SPECIES AGAINST A S. CEREVISIAE WINE STRAIN: COMPETITION AND INFLUENCE IN WINE COMPOSITION

J. Alonso del Real[1], *M. Lairón Peris*[1], *E. Barrio*[2], *A. Querol*[1]
[1]*Instituto de Agroquímica y Tecnología de Alimentos, Biotecnología, Paterna, Spain*
[2]*Universidad de Valencia, Genética, Valencia, Spain*

Backgrounds

Saccharomyces cerevisiae is the main microorganism responsible for the fermentation of wine. Nevertheless, in the last years wineries are facing new challenges due to current market demands and climate change effects on the wine quality. New starters formed by non-conventional *Saccharomyces* species (such as *S. uvarum* or *S. kudriavzevii*) or their hybrids can contribute to solve some of these challenges. They exhibit good fermentative capabilities at low temperatures, producing wines with lower alcohol and higher glycerol amounts. However S. *cerevisiae* can competitively displace other yeast species from wine fermentations.

Objectives

We analyzed the survival capacity of non-*cerevisiae* strains in competition with *S. cerevisiae* during fermentation of synthetic wine must at different temperatures, and the effect on final wine composition.

Methods

We developed a method based on QPCR to quantify the proportion of different *Saccharomyces* yeasts in mixed cultures, which allowed us to assess the effect of competition on their growth fitness. In addition, fermentation kinetics parameters and final wine composition were also analyzed through weight loss and HPLC.

Conclusions

We observed that *S. uvarum* outcompeted *S. cerevisiae* during competences at lower temperatures, as it does in traditional wineries in certain European regions. From an enological point of view, mixed fermentations using some of our species deteriorated the process or the final product composition compared to single *S. cerevisiae* inoculation, whereas fermentation performance and wine characteristics improvements were obtained from other combinations. Finally, wine strains of *S. cerevisiae* and *S. uvarum* performed better in competition than non-wine strains of the same species.

FEMS7-2863
Food Microbiology

EFFECT OF SEVERAL MODIFIED ATMOSPHERE PACKAGING CONDITIONS ON THE SHELF-LIFE OF REFRIGERATED OSTRICH MEAT

R. Capita[1], T. Álvarez-González[1], C. Alonso-Calleja[1]
[1]Universidad de León, Food Hygiene and Technology, León, Spain

Backgrounds

To allow competitive marketing of ostrich meat it is necessary to know its microbial and sensory characteristics under different conditions with a view to prolonging the shelf-life.

Objectives

This study was designed to investigate the effects of eight atmosphere conditions on microbial loads, physicochemical parameters and sensory properties of ostrich steaks during refrigerated storage in order to determine the most effective packaging atmosphere for extending the shelf-life of this foodstuff.

Methods

Ostrich steaks were packaged in air (AIR), vacuum (VAC), MAP1 (70% O_2 + 30% CO_2), MAP2 (30% O_2 + 30% N_2 + 40% CO_2), MAP3 (20% O_2 + 30% N_2 + 50% CO_2), MAP4 (50% N_2 + 50% CO_2), MAP5 (20% N_2 +80% CO_2) or MAP6 (100% CO_2). Microbial counts, pH, Aw and sensory properties (nine-point hedonic scale) were determined on Days 0, 1, 3, 7 and 15 of storage (4 ºC ± 1 ºC).

Conclusions

On Day 0, microbial counts (log_{10} cfu/g) ranged from undetectable levels (*Brochothrix thermosphacta*) to 3.21 ± 0.63 (total aerobic counts, TAC). The highest and the lowest microbial loads throughout storage were observed in AIR and MAP6, respectively. On Day 15 TAC as high as 9.96 ± 0.20 log_{10} cfu/g were found in AIR. The self-life (time until overall acceptability score fell below 5) was 3 (MAP1, MAP2), 7 (MAP3, AIR) or 15 days (MAP4, MAP5, MAP6). Only for VAC the shelf-life limit extended beyond 15 days. Findings in this study offer practical applications to the meat industry for improving the durability of ostrich.

FEMS7-2869
Food Microbiology

MICROBIOLOGICAL QUALITY OF RETAIL RAW MEAT PREPARATIONS IN SPAIN

L. Buzón-Durán[1], S. Blanco-Morán[1], C. Alonso-Calleja[1], R. Capita[1]
[1]Universidad de León, Food Hygiene and Technology, León, Spain

Backgrounds

Meat and meat products constitute a substantial portion of the present-day human diet. To the best of our knowledge, studies on microbiological quality of meat preparations are lacking in north-western Spain.

Objectives

The aim or this work was to assess the loads of various indicator microbial groups in raw meat preparations.

Methods

A total of 160 samples of raw poultry, raw beef and raw pork preparations were collected from retail outlets in the Province of León in north-western Spain. Samples included crepes, escalope, hamburgers, meatballs, minced meat, nuggets, red sausages, and white sausages. Microbiological analysis was performed according to standard methods.

Conclusions

The loads (mean ± STD; log cfu/g) for aerobic plate counts (APC), psychrotrophs, *Enterobacteriaceae*, coliforms, *Micrococcaceae* and enterococci were 6.59 ± 1.10, 6.63 ± 1.19, 2.96 ± 0.98, 1.59 ± 0.92, 4.18 ± 0.92, and 2.07 ± 0.81, respectively. APC were regarded as satisfactory (38 samples; 23.75%), acceptable (46; 28.75%) and unsatisfactory (76; 47.50%) according to the EU microbiological criteria. Both animal species and type of product influenced ($P < 0.05$) microbial counts. Pork preparations showed higher bacterial load than did poultry or beef preparations for most microbial groups. Data (log cfu/g) for APC were 7.06 ± 0.89, 6.42 ± 1.13, and 6.45 ± 1.09, respectively.

The relatively high bacterial counts observed raise concerns regarding the potential risks for public health of meat preparations and suggests that it is vital to design education strategies for food handlers in order to prevent cross-contamination and to ensure that foodstuffs are thoroughly cooked.

FEMS7-2676
Food Microbiology

MODELING GROWTH OF VEROCYTOTOXIGENIC ESCHERICHIA COLI THROUGH RESPONSE SURFACE METHODOLOGY AND ARTIFICIAL NEURAL NETWORKS

M. Prieto-Calvo[1], A. Alvarez-Ordóñez[1], S. López[2], M. López[1], M. Prieto[1]
[1]Universidad de León, Food Hygiene and Technology, León, Spain
[2]Universidad de León, Animal Production, León, Spain

Backgrounds

Quantitative risk assessments are needed to take risk management decisions concerning food-borne microbial hazards. The use of predictive models that describe microbial growth helps estimate the concentration of bacteria in foods.

Objectives

Most models available in the literature predicting verocytotoxigenic *E. coli* (VTEC) growth do not take into account the variability associated to strain diversity. This study analyzed the differences in growth of twelve VTEC strains in several conditions of temperature, acidity and salt concentration by using turbidimetric measurements and modeling techniques.

Methods

The non-linear Baranyi equation was fitted to 1,143 growth curves. Two types of secondary models (response surface methodology (RSM) and artificial neural networks (ANN)) were used to describe the individual and combined effects of temperature, pH and NaCl concentration on kinetic parameters (specific growth rate and lag time).

Conclusions

A decrease in temperature from 35ºC to 25ºC caused a gradual decrease in μ_{max} and increase in λ. pH in the range 6.0 to 7.0 barely affected λ or μ_{max}. However, a pH decrease up to pH 5.0 caused a dramatic decrease in μ_{max} and increase in λ. Finally, NaCl concentrations in the range 0 – 3% had minor effects on growth parameters. RSM yielded good results in relation to goodness of fit, but ANN gave rise to better estimates. Interstrain and interserotype variability was low. In fact, no statistical differences could be observed in growth ability between strains from O157 and non-O157 serotypes, or strains with an impaired RpoS and strains with a functional RpoS.

FEMS7-3198
Food Microbiology

REDUCTION OF TYRAMINE IN FERMENTED FOOD

J. Anderegg[1], A. Die[1], L. Meile[1]
[1]Institute of Food- Nutrition and Health, Laboratory of Food Biotechnology, Zurich, Switzerland

Backgrounds

Tyramine belonging to the biogenic amines (BA) can often be detected in fermented food such as cheese and dry sausages due to endogenous tyrosine decarboxylating microorganisms (MO) and represent a consumer health-risk to be reduced in future.

Objectives

To identify the major tyramine-producing microorganisms in fermented food and to improve the knowledge about the tyramine producing mechanism.

Methods

A variety of cheeses and fermented sausages were screened on different selective agars for different lactic acid bacteria (LAB) and staphylococci. All isolated strains were screened by PCR for the tyramine decarboxylase gene *tyrDC*. Furthermore, an Ion exchange chromatography was developed to determine tyramine and other biogenic amines in food.

Conclusions

We screened 89 cheeses and 21 dry sausages by PCR for *tyrDC* and by IC on biogenic amines. A total of 45 cheeses and 9 dry sausages contained tyramine. In 40% of the cheeses containing tyramine a high content of enterococci between 3.0×10^3 and 3.5×10^7 cfu/g was found. All of these enterococci contained *tyrDC* PCR-amplicons. It seems as if cheeses contains tyramine enterococci plays a role. In sausages it looks different. It seems like enterococci does not becomes an important role in tyramine formation. A more interesting role in tyramine formation in dry sausages seems to have staphylococci, which are used as spontaneous or directed starter culture. Sixteen out of seventeen isolated staphylococci from dry sausages contains *tyrDC* PCR-amplicons. In a next step, the conditions for tyramine production in food will be analyzed.

FEMS7-2954
Food Microbiology

MOLECULAR IDENTIFICATION OF YEASTS AND LACTIC ACID BACTERIA INVOLVED IN THE PRODUCTION OF BENINESE FERMENTED FOOD DEGUE

A.I. Angelov[1], G. Blagoeva[2], A.D. Angelov[1], P. Stefanova[1], I.Y. Bokossa[3], C. Tchekessi[3], M. Petkova[4], V. Gotcheva[1]
[1]University of Food Technologies, Biotechnology, Plovdiv, Bulgaria
[2]Primoris Bulgaria Plc, Microbiological Lab, Plovdiv, Bulgaria
[3]University of Abomey-Calavi, Vegetable Biology, Cotonou, Benin
[4]Agricultural University, Microbiology and Ecological Biotechnologies, Plovdiv, Bulgaria

Backgrounds

Traditional Beninese fermented food Degue is widely consumed in Benin and other countries in West Africa. Nowadays, Degue production occurs by spontaneous fermentation in individual households and information about the microorganisms involved is currently limited. The microbiota in this product from Benin has not been studied so far, but the growing production of Degue in the country sets a demand for revealing the biodiversity of the microbial population involved in the fermentation process in order to take future steps for development of industrial technology and offer products with improved quality and safety.

Objectives

The aim of the present study was to identify the naturally occurring yeast and LAB in cereal raw materials and milk used for the production of Degue, as well as in several types of Degue products.

Methods

In the present study, yeast and lactic acid bacteria (LAB) from raw materials for Degue production and from several Degue products were isolated and identified by molecular methods including RFLP and ITS1-5.8S-ITS2 rRNA gene sequence analysis in yeasts, and 16S rRNA gene sequence analysis in LAB.

Conclusions

Four yeast species were found in the samples of raw materials and various types of Degue - *C. fabianii, C. glabrata*, *K. marxianus* and *M. caribbica,* and identification of the LAB isolates revealed 8 species: *Lb. fermentum*, *Lb. plantarum*, *Lb. pentosus*, *Lb. rhamnosus*, *E. mundtii*, *P. acidilactici*, *S. thermophilus* and *W. paramesenteroides*. The differences of the species diversity in comparison to other similar fermented foods suggest that microbial community structure in Degue is not a simple consequence of the nature of the raw materials used, but it is a result from a variety of abiotic and biotic factors affecting the natural selection of specific microbial populations – origin of the raw materials, climatic and geographical factors, preparation method and work environment, etc.

FEMS7-3181
Food Microbiology

EFFECT OF GLIADIN FRACTIONS ON GROWTH OF PROBIOTIC BACTERIA GENUS LACTOBACILLUS IN VITRO

R. Antolovic[1], *M. Ozbolt*[2], *T. Mančuškova*[3]
[1]*Catholic University of Croatia, Department of Nursing, Zagreb, Croatia*
[2]*University of Rijeka, Department of Biotechnology, Rijeka, Croatia*
[3]*Slovak University of Technology-, Faculty of Chemical and Food Technology, Bratislava, Slovak Republic*

Backgrounds

Incomplete digestion of gliadin in the gastrointestinal tract leads to formation of toxic peptides which can cause immune response in individuals. However, studies have shown that administration of probiotic supplements may prevent or mitigate adverse effects of these peptides. Among probiotic supplements, ones containing probiotic bacteria of genus *Lactobacillus* are standing out a potential therapy.

Objectives

The influence of gliadin on the proliferation of probiotic bacteria has been studied on seven *Lactobacillus* strains *in vitro,* studying two parameters - the growth rate and the lag phase of *Lactobacillus* strains.

Methods

Gliadin used in the sudy was disolved in 70% ethanol in 50 mM Tris/HCl or in 2% sodium chloride solution.

Conclusions

Results show that gliadin dissolved in 70% ethanol and 50 mM Tris/HCl induced proliferation of *L. helveticus* XA/2, *L. plantarum* II/5, *L. rhamnosus* GG and *L. paracasei spp. paracasei* 1753 and had no significant effect on growth of *L. acidophilus* 145, *L. casei* VIIB/6, *L. paracasei* VIIB5. Results with gliadin dissolved in 2% sodium chloride show that gliadin stimulated proliferation of *L. helveticus* XA/2 and *L. plantarum* II/5, but didn't have a significant effect on growth on *L. acidophilus* 145, *L. rhamnosus* GG, *L. casei* VIIB/6, *L. paracasei* VIIB5, *L. paracasei spp. paracasei* 1753. The growth rate of *L. helveticus* XA/2 and *L. plantarum* IIA/5 was higher with increasing concentration of gliadin in solution of 2% NaCl, but the lag fase was also longer because the bacteria needed more time to adjust to new conditions.

FEMS7-2386
Food Microbiology

IMPORTED POULTRY MEAT PRODUCTS IN PORTUGAL AS A SOURCE OF EXTENDED-SPECTRUM CEPHALOSPORIN-RESISTANT SALMONELLA HEIDELBERG CMY-2-PRODUCING

J. Campos[1], J. Mourão[1], L. Silveira[2], M. Saraiva[3], C. Belo Correia[4], A.P. Maças[5], P. Antunes[6]
[1]UCIBIO/REQUIMTE., Laboratório de Microbiologia- Faculdade de Farmácia- Universidade do Porto., Porto, Portugal
[2]Instituto Nacional de Saúde Doutor Ricardo Jorge, Laboratório Nacional de Referência de Infeções Gastrintestinais- Departamento de Doenças Infecios as, Lisboa, Portugal
[3]Instituto Nacional de Saúde Doutor Ricardo Jorge, Departamento de Alimentação e Nutrição, Porto, Portugal
[4]Instituto Nacional de Saúde Doutor Ricardo Jorge, Departamento de Alimentação e Nutrição, Lisboa, Portugal
[5]Direção Geral de Alimentação e Veterinária, Direção Geral de Alimentação e Veterinária, Lisboa, Portugal
[6]Faculdade de Ciências da Nutrição e Alimentação, Faculdade de Ciências da Nutrição e Alimentação, Porto, Portugal

Backgrounds

Extended-spectrum cephalosporin-resistant (ESC-R) *Salmonella* has been described at low level in EU, including Portugal. Nevertheless, the increasing poultry meat trade involving countries with different animal production practices could be an important source of epidemic clones carrying ESC-R genes.

Objectives

To evaluate ESC resistance and to detail genetic platforms as well as the clonal relatedness of *Salmonella* isolates from Brazilian poultry meat products imported into Portugal

Methods

Seven *Salmonella* isolates from two serotypes (n=6-Heidelberg; n=1-Minnesota) recovered from frozen gizzards imported from Brazil (2014-2015) were studied. Antibiotic susceptibility tests (ampicillin-A/chloramphenicol/ciprofloxacin-Cip/gentamicin/kanamycin/meropenem/nalidixic acid-Na/pefloxacin-P/streptomycin/sulfamethoxazole-Su/tetracycline-T/trimethoprim) and detection of beta-lactamase production (amoxicillin-clavulanic acid-Amc/cefepime/ceftazidime-Cz/cefotaxime-Cx/cefoxitin-Fx) were performed by disk diffusion/microdilution methods (CLSI/EUCAST). Detection of genes encoding for acquired AmpC beta-lactamases (*bla*$_{CMY}$/*bla*$_{MOX}$/*bla*$_{FOX}$/*bla*$_{LAT}$/*bla*$_{ACT}$/*bla*$_{MIR}$/*bla*$_{DHA}$/*bla*$_{ACC}$), extended-spectrum beta-lactamases (*bla*$_{TEM}$/*bla*$_{SHV}$/*bla*$_{CTX-M}$), plasmid-mediated quinolone resistance [*qnrA*/*qnrB*/*qnrC*/*qnrD*/*qnrS*/*qepA*/*aac(6')-Ib-cr*/*oqxAB*] and other antibiotic resistance (PCR/sequencing), plasmid characterization (PCR-PBRT/pMLST/sequencing), conjugation assays and genomic location (I-CeuI/S1-PFGE nuclease hybridization) were performed. Clonal analysis by XbaI-PFGE/MLST included comparison with Portuguese/EU clinical/foodborne strains.

Conclusions

All imported poultry isolates were MDR (mostly AAmcCzCxFxCpPNaSuT), including to extended-spectrum-cephalosporins (MIC$_{Cx}$=8-16mg/L/MIC$_{FX}$=32->256mg/L) and/or fluoroquinolones (MIC$_{Cip}$=0.25-0.5mg/L). All but one isolate carried *bla*$_{CMY-2}$ located on two plasmid-types (IncA/C/ST2; n=4-Heidelberg/ST15; n=1-Minnesota/ST548; IncI1/ST12; n=1-Heidelberg/ST15). *S*.Heidelberg was

associated with 6 PFGE-types, with Brazilian poultry isolates sharing identical PFGE-types with a Portuguese patient or poultry imported into The Netherlands. Introduction in Portugal of CMY-2, mostly associated with MDR *S.*Heidelberg ST15 clone, emergent in American countries, suggest an important role of international food trade for the occurrence of ESC-R in EU. Furthermore this raises questions about the Regulation (EU) Nº1086/2011 setting for fresh poultry meat a criterion covering only *S.*Enteritidis and *S.*Typhimurium.

FEMS7-1794
Food Microbiology

DISTRIBUTION OF TOP-EIGHT PATHOGENIC O-SEROGROUPS IN SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) STRAINS ISOLATED FROM DIFFERENT ANIMAL SOURCES IN IRAN

M. Askari Badouei[1], *H. Taban*[2], *H. Ajoudanifar*[2]
[1]*Garmsar Branch- Islamic Azad University, Pathobiology, Garmsar, Iran*
[2]*Damghan Branch- Islamic Azad University, Microbiology, Damghan, Iran*

Backgrounds

Shiga toxin- producing *Escherichia coli* (STEC) have been considered as one of the most important food-borne pathogens worldwide. Although a wide range of *E. coli* serogroups has been implicated in human infections, most severe cases in Europe and the United States were related to certain serogroups known as top-eight STEC serogroups.

Objectives

Lack of the availability of serotyping data in most developing countries including Iran has been a public health challenge, especially to track outbreaks and to monitor the possible sources. Therefore, we aimed to investigate the distribution of major STEC serogroups in a large collection of STEC strains isolated from different provinces and variety of sources for the first time in Iran.

Methods

: Total of 118 non-duplicate STEC strains isolated in previous studies was selected. The isolates were obtained from cattle (35), sheep (25), goats (35), and pigeons (23). All isolates were subjected to a multiplex - PCR assay detecting the major virulence genes (*stx1, stx2, stx2f, eae, Ehly*) and then tested for top- eight O-groups including O26, O45, O103, O111, O113, O121, O145, and O157 by PCR.

Conclusions

The predominant serogroup was O113 as it was detected in 5 cattle and 3 sheep and goats strains. It was followed by O26 and O111 each occurred in 3 isolates from the cattle group, respectively. The pigeon isolates did not belong to any of the tested serogroups.

FEMS7-0630
Food Microbiology

EVALUATION OF GREEN TEA EXTRACT FOR INACTIVATION OF ENTERIC VIRUSES IN FOOD APPLICATIONS

R. Aznar[1,2], I. Falcó[1], W. Randazzo[1], G. Sanchez[1,2]
[1]Universitat de Valencia, Microbiología y Ecología, Valencia- Burjassot, Spain
[2]Institute of Agrochemistry and Food Technology IATA-CSIC, Food Safety and Preservation, Burjassot, Spain

Backgrounds

Green tea extract (GTE) is a polyphenolic and proanthocyanidin rich compound widely used in food and beverage applications with health benefits including antioxidant, anti-inflammatory, and anticarcinogenic properties. In addition, GTE demonstrated antimicrobial activity against Gram-positive and Gram-negative foodborne bacterial pathogens and it has been used as a component in multiple hurdle approaches to enhance food safety and quality.

Objectives

To evaluate the effect of GTE on the infectivity of murine norovirus (MNV) and hepatitis A virus (HAV), and its application as disinfectant in vegetables and food-contact surfaces.

Methods

Virus suspensions (5 log $TCID_{50}$/ml) were incubated for 2 h in the presence of GTE (at 0.5 and 5 mg/ml): a) at pH ranging from 5.5 to 8.5 and 37 °C; b) at 4, 25 and 37 °C and pH 7.2. GTE was evaluated under food industry simulated conditions (ISO 13697:2001) on stainless steel and glass surfaces, lettuce and spinach artificially inoculated with viral suspensions. Antiviral activity was evaluated by cell-culture.

Conclusions

GTE affected both viruses with higher reductions at alkaline pH. Complete inactivation in suspension tests was achieved after overnight exposure at 37 °C for both viruses and also at 25 °C for HAV.

GTE (10 mg/ml) proved efficient after 30 min surface disinfection, since 1.5 log reduction and complete inactivation were recorded for MNV and HAV on stainless steel and glass surfaces, respectively. As a natural disinfectant, GTE (10 mg/ml) reduced MNV and HAV titers in lettuce and spinach by more than 1.5 log after 30 min treatment.

FEMS7-1349
Food Microbiology

DEVELOPMENT OF KEFIR-LIKE CEREAL-BASED BEVERAGE NATURALLY BIO-FORTIFIED IN RIBOFLAVIN

R. Aznar[1], A. Yépez[1], P. Russo[2], V. Capozzi[2], G. Spano[2]
[1]Universitat de Valencia, Microbiología y Ecología, Valencia- Burjassot, Spain
[2]University of Foggia, Department of the Science of Agriculture- Food and Environment, Foggia, Italy

Backgrounds

Lactic acid bacteria can be used in fermented beverages to enrich their nutritional value, either by vitamins production or increasing nutrients bioavailability. Kefir is a fermented milk that has become very popular because its healthy attributes. The development of cereal-based functional beverages is particularly attractive to avoid dairy intolerances and to use matrices with recognized functional properties. While cereal-based yogurt-like beverages have been developed, scarce information is available about kefir-like products formulated from cereal flours.

Objectives

To develop a kefir-like cereal-based product and to select and inoculate a vitamin overproducer *Lactobacillus plantarum* strain to increase riboflavin content of the fermented beverage.

Methods

Six riboflavin-producer *L. plantarum* strains were evaluated for industrial application by testing growth at different pH, temperature and salinity as indicators of technological stress tolerance. In addition, they were exposed to roseoflavin to obtain riboflavin over-producing mutants. Fermentation of barley, corn and oat infusions was carried out using two commercial kefir starters together with the addition of a riboflavin over-producing strain (selected among the six on the basis of technological stress tolerance and of the over-producing phenotype). Physico-chemical parameters (lactic and acetic acids, CO_2, ethanol and, viscosity) and riboflavin content were monitored during the process and in the final products.

Conclusions

To the best of our knowledge, we report for the first time the design of cereal-based kefir-like beverages. Three novel non-dairy kefir-like products were developed and characterized. Beverages were produced inoculating a riboflavin over-producing *L. plantarum* strain (together with starter cultures) in order to increase final vitamin B_2 content.

FEMS7-1454
Food Microbiology

THE INFLUENCE OF COLD STRESS ON GENE EXPRESSION IN SHIGA TOXIN-PRODUCING ESCHERICHIA COLI ON ROMAINE LETTUCE

S. Bach[1], C. Harlton[1], M. Gardiner[1], M. Pylatuk[1], M. Kostrzynska[2]
[1]Agriculture and Agri-Food Canada, Science and Technology Branch, Summerland, Canada
[2]Agriculture and Agri-Food Canada, Science and Technology Branch, Guelph, Canada

Backgrounds

Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) have emerged as important human pathogens being implicated in cases of foodborne illness associated with leafy greens. A better understanding of the fitness and stress response of non-O157 STEC is necessary in order to design effective mitigation strategies for their control.

Objectives

The objective of this study was to examine the effect of cold temperature on expression of stress and virulence genes in six STEC strains.

Methods

Logarithmic phase cultures (10^8 CFU/ml) of *E. coli* O103:H2, O26:H11, O111:NM, O157:H7, O145:NM and O104:H4, each stressed at 4 °C for 5 d, were used as inoculum in: 1) STEC + Lettuce (SL); 2) STEC Inoculum, no lettuce (I). Lettuce only was used as a control. Duplicate samples (25 g) of 4 x 10 cm pieces were prepared for each biological sample (n=3, lettuce heads). Expression of virulence (*eae*, *stx1*, *stx2*) and stress genes (*uspA*, *rpoS*, *phoA*, *dps*, *cspA*, *cspC*, *cspE*, *mutS*, *gadW*) was examined using a two-step reverse-transcriptase comparative quantitative real-time PCR. Data was analyzed using Biogazelle's qbasePLUS (ANOVA, $P<0.05$).

Conclusions

CspA was upregulated ($P<0.05$) and *cspC* and *uspA* were downregulated ($P<0.05$) in all serotypes, while *cspE* was not differentially expressed. Cold stress resulted in down-regulation of *dps* in all serotypes except O145:NM. *Stx1A* was differentially expressed among serotypes in which the gene was present (P>0.05). Results provide an increased understanding of the variable cold temperature acclimatization exhibited by O157 and non-O157 STEC, providing knowledge which can be used to develop effective control strategies.

FEMS7-0794
Food Microbiology

IMPROVED RADICAL-SCAVENGING, METAL ION-REDUCING, AND EX VIVO ANTIOXIDANT ACTIVITY OF WATER EXTRACT OF INULA BRITANNICA FERMENTED BY LACTOBACILLUS PLANTARUM IN HEPG2 CELLS

W.Y. Bae[1], H.Y. Kim[1], K.T. Kim[2], H.D. Paik[1]
[1]Konkuk University, Food Science and Biotechnology of Animal Products, Seoul, Republic of Korea
[2]Konkuk University, Bio/Molecular Informatics Center, Seoul, Republic of Korea

Backgrounds

Inula britannica extract is known to have strong anti-wrinkle effect inhibited by intracellular tyrosinase.

Objectives

The aim of this study was to measure the anti-oxidative activity of a water extract of *I. britannica* fermented by *Lactobacillus plantarum*.

Methods

Radical scavenging assays detected reduced amounts of DPPH$^{\bullet}$, ABTS$^{\bullet+}$, O_2^-, and $^{\bullet}$OH following treatment with the extract. In ABTS radical scavenging assay, antioxidant activity was evaluated based on trolox equivalent antioxidant capacity (TEAC). Metal ion-reducing ability was determined by measuring the reduction of Fe^{3+} and Cu^{2+}. Chelating abilities were evaluated by Fe^{2+}-ferrozine and Cu^{2+}-pyrocatechol violet complex. Cell cytotoxicity and anti-oxidative effect of *I. britannica* extracts in HepG2 cells were determined by the MTT assay and the Enzychrom™ assay kit, respectively.

Conclusions

Fermented *I. britannica* extract had more than 80% radical scavenging activity at a concentration of 500 μg/mL ($p<0.05$). According to an ABTS$^{\bullet+}$ assay, the fermented extract had 85.08–694.25 μM/mL TEAC, whereas the non-fermented control had 59.67–477.17 μM/mL TEAC. Metal ion-reducing abilities were more effective in the fermented extract than those in the control ($p<0.05$), but chelating abilities were more effective in non-fermented extract. The extract had no measurable cell cytotoxicity up to 500 μg/mL in HepG2 cells. The levels of SOD, GPx, catalase, and glutathione in HepG2 cells treated with the fermented extract were increased compared to cells treated with the non-fermented extract. These results suggest that fermentation increased the antioxidant activity of *I. britannica* extract, which could be applied as a natural antioxidant in the food and cosmetic industries.

FEMS7-0844
Food Microbiology

ANTIMICROBIAL ACTIVITY OF INULA BRITANNICA EXTRACT FERMENTED BY LACTOBACILLUS PLANTARUM

W.Y. Bae[1], H.Y. Kim[1], K.T. Kim[2], H.D. Paik[1]
[1]Konkuk University, Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea
[2]Konkuk University, Bio/Molecular Informatics Center, Seoul, Republic of Korea

Backgrounds

Inula britannica is a wild plant in eastern Asia and has been used as a traditional medicine for bronchitis and digestive disorders.

Objectives

The aim of this study was to investigate the antimicrobial activity of water extract of *I. britannica* fermented by *Lactobacillus plantarum* against food-borne pathogens.

Methods

The antimicrobial activity of *I. britannica* extract was measured against 15 strains of food-borne pathogens by disc diffusion assay. Cell membrane integrity was determined by measuring leakage of nucleic acids and proteins, and membrane potential was evaluated using Rhodamine 123. In a time-kill assay, the inhibitory effects of the fermented extract on cell growth of *Bacillus cereus* and *Listeria monocytogenes* were performed in broth containing fermented extract at concentrations of 0.5×MIC, 1×MIC, or 2×MIC.

Conclusions

In a disc diffusion assay, discs treated with the fermented extract had inhibition zone in *B. cereus* (4 stains) and *L. monocytogenes* (4 strains), and the MIC values were 2.5-7.5 mg/mL. The membrane integrity of treated cells was significantly different from that of the control cells, at all concentrations of extract ($p<0.05$). For both *B. cereus* and *L. monocytogenes*, the membrane potential of cells treated with 1×MIC of the fermented extract was significantly different ($p<0.05$) from that of the untreated cells. A time kill assay determined that the fermented extract had bactericidal activity at 2×MIC and bacteriostatic activity at 1×MIC against *B. cereus* and *L. monocytogenes*. These results suggest that fermented water extract of *I. britannica* has antimicrobial activity and potential applications as a natural food preservative in food processing.

FEMS7-2493
Food Microbiology

MICROBIOLOGICAL SURVEY OF RAW MILK FROM DAIRY PROCESSING UNITS IN SAVADKOOH REGION

B. Saadatmand[1], A. Bapiri[2]
[1]M.Sc.Graduated Student of Food Science and Technology- Department of Agriculture- Savadkooh B ranch- Islamic Azad University, ., Savadkooh, Iran
[2]Department of Agriculture- Savadkooh Branch- Islamic Azad University, ., Savadkooh, Iran

Backgrounds

In the Savadkooh region, the production of milk and dairy products is affected by the application of improper procedures during milking and processing, leading to microbial spoilage of raw milk, especially in local small scale processing plants.

Objectives

The aim of this survey was to identify potential hazards and evaluate the microbial contamination of raw milk in two dairy processing units located in Savadkooh.

Methods

At the beginning of April, May, June, July and August, raw milk samples were collected from processing units and transported to the laboratory. During 5 months period, raw milk samples were evaluated for total microbial load, total Coliform and *Escherichia coli* in accordance with the standards No. 356, 2852 and 2629.

Conclusions

The results showed that contamination levels of raw milk samples varied widely. The total microbial load was April the highest (2×10^4 CFU/ml) and August the lowest (7×10^3 CFU/ml) but total Coliform was the highest (7.85%) in August and (0.00%) in June. E.coli contamination were not observed in any of the samples. Based on microbiological outcomes and milk production flow characterization, preventive and corrective measures were defined for milking and processing phases, focusing on training of farmers and dairy employees to improve the hygiene of the local milk and dairy production chain.

FEMS7-1780
Food Microbiology

INFLUENCE OF PHYSICAL AND CHEMICAL CHARACTERISTICS OF WINE GRAPES ON THE INCIDENCE OF PENICILLIUM AND ASPERGILLUS FUNGI IN GRAPES AND OCHRATOXIN A IN WINES

L. Freire[1], F. Passamani[1], A. Thomas[1], R. Nassur[1], L. Silva[1], F. Paschoal[2], G. Pereira[3], G. Prado[2], L. Batista[1]
[1]Federal University of Lavras, Food Science, Lavras, Brazil
[2]Fundação Ezequiel Dias, Mycotoxin Lab, Belo Horizonte-MG, Brazil
[3]EMBRAPA SEMI-ÁRIDO, Pós-Colheita, Petrolina-PE, Brazil

Backgrounds

The incidence of filamentous fungi and toxin levels in grapes and wines varies depending on the variety of grapes, the wine region, agricultural practices, weather conditions, the harvest and the winemaking process.

Objectives

The objective of this study was to evaluate the diversity of *Aspergillus* and *Penicillium* fungi isolated from wine grapes of the semi-arid tropical region of Brazil, evaluate the presence of ochratoxin A (OTA) in the experimental wine and verify if there is a correlation between occurrence of these fungi and the physicochemical characteristics of the wine grapes grown in the region.

Methods

For the isolation of fungi we used the direct plating technique. The presence of OTA in the experimental wine was detected by high-performance liquid chromatography.

Conclusions

The species found were *Aspergillus niger*, *A. carbonarius*, *A. aculeatus*, *A. niger* Aggregate, *A. flavus*, *A. sojae*, *Penicillium sclerotiorum*, *P. citrinum*, *P. glabrum*, *P. decumbens*, *P. solitum* and *P. implicatum*. All isolates of *A. carbonarius* were OTA producers and all *P. citrinum* were citrinin producers. The highest concentration of OTA was found in red wine (0.29 µg/L). All species identified in this study, except *A. flavus*, showed a positive correlation with at least one physicochemical parameter assessed, highlighting the pectin content, total sugar, total acidity and phenolic compounds.
Financial Support: FAPEMIG, CNPq.

FEMS7-1833
Food Microbiology

CULTURE COLLECTION OF DEPARTMENT OF FOOD SCIENCE (CCDCA), FEDERAL UNIVERSITY OF LAVRAS, BRAZIL

L. Batista[1], M. Terra[1], N. Lira[1], F. Passamani[1], S. Chalfoun[2], J. Machado[1], D. Botrel[1], J. Teodoro[1]
[1]Federal University of Lavras, Food Science, Lavras, Brazil
[2]EPAMIG, Coffee Quality Control, Lavras, Brazil

Backgrounds

Brazil is a country with one of the highest levels of biological diversity due to its large geographical area, high coverage by rainforests, and endemic biomes, such as Brazilian Atlantic Forest, Cerrado and Caatinga. Much of this unknown biodiversity is in the tropics, which is seriously threatened by some agricultural activities, reinforcing the urgent need for biodiversity studies in these areas.

Objectives

The aims of CCDCA are: a) preserve *Aspergillus* and *Penicillium* strains for comparative studies between Brazilian regions and worldwide; b) promote the study of *Aspergillus* and *Penicillium* for (i) biotechnological and toxigenic potential, and (ii) training in identification, preservation and distribution; c) establish quality control of fungal assays; and, finally, d) establish the CCDCA as a member of the global network of culture collections and participate in the international forums and organizations related to culture collections.

Methods

For the identification of the isolates are used techniques proposed by specialized literature, such as specific culture media and identification manuals. All fungal isolates belonging to the CCDCA collection are preserved by the filter paper method in three conditions: ultrafreezer at -80ºC, refrigerator at 4ºC and ambient temperature.

Conclusions

Since then, it has been modernized, monitored and maintained financially through research projects. Currently the CCDCA collection consists of 735 isolates belonging to the genus *Aspergillus* and *Penicillium*, obtained from various substrates, including food, agricultural products, cultivated land, wastewater, agro-industrial waste, mineral exploitation soils, grapes, coffee, cocoa and industrialized products such as cheeses, chocolate, sweets and beverages. The CCDCA is member of WDCM 1081 and WFCC.
Fiancial Support: FAPEMIG and CNPq (PROTAX).

FEMS7-1213
Food Microbiology

IN THE SUBTELOMERIC-DAWN OF SACCHAROMYCES' CHROMOSOME VI

I. Belda[1], *J. Ruiz*[1], *L.J. López-Iglesias*[2], *D. Marquina*[1], *A. Acedo*[2], *A. Santos*[1]
[1]*Biology Faculty- Complutense University of Madrid, Microbiology, Madrid, Spain*
[2]*Biome Makers, WineSeq, San Francisco, USA*

Backgrounds

Genetic recombination, a process in which exogenous DNA is acquired and incorporated into the genome, is a key evolutionary mechanism for several microorganisms. At the megabase scale, the average recombination rate is higher in the subtelomeric regions than in the middle part of chromosomes. Some *Saccharomyces cerevisiae* wine strains showed an introgressed segment (derived from *S. paradoxus*) covers over the last 15 kb near the telomere of chromosome VI. In this context, we observed distinctive metabolic features associated to this event, such as a reduced fitness and differential response to nutrient starvation.

Objectives

The aim of this work is to characterize the chromosome VI subtelomere region in *S. cerevisiae* wine strains, to understand the phenotypic features associated to its different versions.

Methods

Using Whole-Genome-Sequencing (Illumina-MiSeq platform) we obtained the entire genome sequence of 9 wine yeast strains. Genomes were assembled using conventional bioinformatics software (edena, spades…); and public online resources (GenBank, BLAST and CLUSTAL-O) were used to explore the genomic variability of the ORFs contained in this region.

Conclusions

Only an 8% of *S. cerevisiae* wine strains showed the exogenous introgressed version in the subtelomere of chromosome VI. This version includes a longer allele of *IRC7* gene (codifying for a high-active β-lyase enzyme, with enological interest). Relevant genes such as *HXK1*, *RET2* or *RPN12* are close-located to *IRC7*. We are now exploring the existence of non-competitive polymorphisms of these genes, that could be on the basis of their low abundance in wild strains and contributing to the distinctive behavior of this intraspecific clade.

FEMS7-1889
Food Microbiology

GENETIC TYPING AND PHENOTYPIC VIRULENCE FACTORS OF DEBARYOMYCES HANSENII ISOLATED FROM FOOD

C. Belloch[1], *R. Montero*[1], *M. Flores*[1]
[1]*Institute of Agrochemistry and Food Technology, Biotechnology, Paterna Valencia, Spain*

Backgrounds

Phenotypic virulence factors are usually tested on yeasts from clinical origin but not on yeast from food origin. However, several previous studies have found yeast food isolates pertaining to the species *D. hansenii* to be potentially pathogenic. These findings may limit the use of D. hansenii as starter in food to only those strains that exhibit none of few pathogenic traits described in yeasts.

Objectives

In this study, a collection of *D. hansenii* yeasts isolated from meat fermented sausages, matured cheese and fermented vegetables were screened to be used as starters in food fermentation. First, we carried out a genetic typing of the strains which would allow monitoring of starter growth in a fermentative process and its discrimination from wild yeasts. In a second step, the strains were screened for several traits associated with the potential pathogenicity of yeasts.

Methods

Genetic typing was carried out by sequencing of the ITS1-5.8S-ITS2 rDNA region and ACT1 gene as well as RAPDs of minisatellite M13 and inter-LTR PCR fingerprinting. Among the fenotypic virulence factors growth at different temperatures, ability to produce biofilm, pseudohyphal and invasive growth and production of phospholipases, proteases, esterases and haemolysins were tested.

Conclusions

Out results showed that very few *D. hansenii* strains from food origin have the potential to be pathogenic. Sequencing of ACT1 probed to be more discriminating than sequencing of ITS1-5.8S-ITS2 rDNA. However, results produced by sequencing could not reach the level of discrimination generated by PCR amplification using M13 minisatellite primers (RAPDs-M13) or inter-RTL PCR fingerprints.

FEMS7-2511
Food Microbiology

ANTIMICROBIAL PROPERTIES AND MEMBRANE INSERTION OF PLANTARICIN149 ANALOGUES

J.L.S. Lopes[1], P.S. Kumagai[2], I.L.B.C. Camargo[2], J. Burck[3], B.A. Wallace[4], L.M. Beltramini[2]
[1]USP - IF, Applied Physics, Sao Paulo, Brazil
[2]USP, IFSC, Sao Carlos, Brazil
[3]KIT, KIT, Karlsruhe, Germany
[4]Birkbeck, Cristallography, London, United Kingdom

Backgrounds

Antimicrobial peptides (AMP) produced by lactic acid bacteria are of particular interest in food industry due to their potential application as food biopreservative, and also useful for pharmaceutical industry, since AMPs constitute the first line of defence against invading bacteria, viruses, and fungi. Synthetic Plantaricin149 (Pln149) is a member of this group of cationic AMPs with inhibitory activity against *Staphylococcus aureus*, *Listeria* and *Saccharomyces cereviseae*, causing membrane destabilization via a initial electrostatic interaction with charged lipids.

Objectives

In order to better understand the mechanism of action and plan more active AMPs, a series of Pln149 analogues with modifications at their N-terminus were designed and used to modulate the antimicrobial properties and peptide insertion in model membranes.

Methods

Pln149-analogues inhibitory action were evaluated against pathogenic bacteria by MIC determination, haemolytic activity and correlated to measurements of peptide binding and orientation in different lipid systems, using oriented synchrotron radiation circular dichroism spectroscopy.

Conclusions

Improved bacterial activity was achieved on Pln149 by increasing the non-polar nature (adding Fmoc groups) at its N-terminus. Fmoc-Pln149 had inhibitory activity against *S. epidermidis* ATCC35984*, S. aureus* ATCC25923*, E. faecalis* ATCC29212, *E. faecium* VRE16*, K. pneumoniae* ATCC700603*, E. coli* ATCC25922 (>512 ug/mL) and showed low red blood cells lysis degree. During binding to negatively charged phospholipids, all analogues assume a helix structure which disturbs the packing of lipid bilayers with its insertion parallel to plane of the membrane surface The characterization of more active AMPs could represent an alternative process in the search of powerful antibacterial agents.

FEMS7-2462
Food Microbiology

COMBINE USE OF SCHIZOSACCHAROMYCES POMBE AND LACHANCEA THERMOTOLERANS YEAST STRAINS AS AN ALTERNATIVE TO THE TRADITIONAL MALOLACTIC FERMENTATION IN WINE PRODUCTION

S. Benito[1]
[1]*Polytechnic University of Madrid, Chemistry and Food Technology, Madrid, Spain*

Backgrounds

Most red wines commercialized in the market use the malolactic fermentation process in order to ensure stability from a microbiological point of view. In this second fermentation, malic acid is converted into L-lactic acid under controlled setups. However this process is not free from possible collateral effects that on some occasions produce off-flavors, wine quality loss and human health problems.This biotechnology develops a new red winemaking methodology that consists of combining the use of two non-Saccharomyces yeast strains as an alternative to the traditional malolactic fermentation. In this method, malic acid is totally consumed by Schizosaccharomyces pombe, thus achieving the microbiological stabilization objective, while Lachancea thermotolerans produces lactic acid in order not to reduce and even increase the acidity of wines produced from low acidity musts. The result is more fruity wines that contain less acetic acid and biogenic amines than the traditional controls.

Objectives

To introduce a new biotechnology to produce wine different from classical ones

To get higher quality wines

To get safer wines from a food safety point of view (lower content in histamin)

To show the results of last two years research about the topic from experimental winery of Madrid Polytechnic University.

Methods

Microvinifications at laboratory scale from 50 mL to 100 mL

Industrial vinifications (winemaking) from 500 L to 20000 L at real winery scale (university winery)

HPLC (anthocyanins profile, amino acids profile, biogenic amines profile)

GC/MS (wine aroma profile)

Regular chemical analyses of wine

Conclusions

The combination of the non-Saccharomyces Lachancea thermotolerans and Schizosaccharomyces pombe positively influenced wine quality.

New fermentations produced higher levels of glycerol and pyruvic acid.

New fermentations produced reduced ethanol levels.

New fermentations produced wines stabilised from a malic acid point of view without any need of performing a malolactic fermentation.

Wines showed lower final levels of biogenic amines (mainly histamine).

FEMS7-3111
Food Microbiology

INVESTIGATION OF THE INOCULATION TIME OF APULIAN AUTOCHTHONOUS OENOCOCCUS OENI AND LACTOBACILLUS PLANTARUM STRAINS IN IN MULTI-STARTER WINE FERMENTATIONS

C. Berbegal[1], L. Beneduce[2], A. Vernile[2], F. Grieco[3], G. Spano[2], V. Capozzi[2]
[1]University of Foggia, Department of Sciences of Agriculture- Food and Environment, Foggia, Italy
[2]University of Foggia, Department of the Sciences of Agriculture- Food and Environment, Foggia, Italy
[3]ISPA, Science of Food Production, Lecce, Italy

Backgrounds

The winemaking process includes two key steps carried out by microorganisms, i.e. alcoholic fermentation (AF) and malolactic fermentation (MLF). The AF primarily produce the conversion of grape must sugars into ethanol. This fermentation is carried out by yeast, mainly by *Saccharomyces cerevisiae* strains: However, this is not the only yeast involved in the process. In fact, several non-*Saccharomyces* species, such as *Hanseniaspora uvarum*, have been proved to positively modify the wine chemical composition, contributing to the sensory characteristics of wines. On the other side, MLF is carried out by lactic bacteria (LAB), principally by *Oenococcus oeni* and *Lactobacillus plantarum* strains.

Objectives

The objective of this work was to identify the best inoculation time of autochthonous of *Oenococcus oeni* or *Lactobacillus plantarum* strains in combination with two autochthonous *S. cerevisiae* strains and one *H. uvarum* strain, all isolated from Apulian wines.

Methods

S. cerevisiae and non-*Saccharomyces,* were co-inoculated in red must, and *L. plantarum* or *O. oeni* strains were co-inoculated or sequentially inoculated during AF, when ethanol content was 2%, 4%, 6%, 8%, 10% or 12% (v/v). Ethanol formation, L-malic acid consumption and bacterial cell viability were monitored during the vinification.

Conclusions

Ethanol concentration at the moment of bacterial inoculation was a crucial factor for the success of MLF. The co-inoculation with *S. cerevisiae* and *H. uvarum* was the best strategy for maintaining the highest LAB concentration, in order to successfully carry out the MLF in red must. Bacterial cell viability and L-malic consumption after AF were strain-dependent (Aupulian Region Grant: QCBRAJ6, 9OJ4W81, LPIJ9P2).

FEMS7-3172
Food Microbiology

OXIDATIVE AND ACIDIC STRESS RESPONSE OF EXOPOLYSACCHARIDES-PRODUCING LACTOBACILLUS PLANTARUM LP90

C. Berbegal[1], A. Longo[2], M.P. Arena[2], D. Fiocco[3], V. Capozzi[2], G. Spano[2]
[1]University of Foggia, Department of Sciences of Agriculture- Food and Environment, Foggia, Italy
[2]University of Foggia, Department of the Sciences of Agriculture- Food and Environment, Foggia, Italy
[3]University of Foggia, Department of Clinical and Experimental Medicine, Foggia, Italy

Backgrounds

Exopolysaccharides (EPS) are long chains of repeating units of sugar or sugar derivatives released outside of the microbial cell wall. *Lactobacillus plantarum* is one of the most widespread lactic acid bacteria (LAB) species presenting different ecological niches. *L. plantarum* Lp90 is a strain isolated from Apulian (South of Italy) wine and has been characterized for its ability to overproduce EPS, which confers a distinctive ropy phenotype. Although this strain is well characterized (we sequenced the genome and studied different probiotic and technological properties), few studies have been carried out on the significance of the exopolysaccharides responsible of the ropy phenotype in the abiotic stress response of this strain.

Objectives

The objective of this work was to investigate the tolerance of the ropy strain *L. plantarum* Lp90 and the non-ropy mutant strain *L. plantarum* Lp90Δ*cps2* exposed to acidic and oxidative stress.

Methods

L. plantarum Lp90 and the non-ropy mutant strain *L. plantarum* Lp90Δ*cps2* were growth in MRS broth for 16 h and, then, suspended in MRS adjusted to pH 2.5 (with HCl 1M) and in saline solution (0.85% NaCl) with SO_2 70 mg L^{-1}. After 30 min of incubation a 37°C, aliquots were removed and plated onto MRS agar in order to estimate viable cells.

Conclusions

The results showed a higher tolerance to acidic and oxidative stresses by the ropy strain *L. plantarum* Lp90 compared to the non-ropy mutant strain *L. plantarum* Lp90Δcps2, which lacks in cps2 cluster involved in the exopolysaccharides biosynthesis, supporting the protective role of exopolysaccharides (Aupulian Region Grant QCBRAJ6, 9OJ4W81).

FEMS7-1422
Food Microbiology

USE OF ATR-FTIR AND FT-RAMAN SPECTROSCOPY COMBINED WITH MULTIVARIATE ANALYSIS TO DISCRIMINATE STRESS-RESISTANT VARIANTS OF ESCHERICHIA COLI MG1655

D. Berdejo[1], L. Espina[1], R. Pagán[1], D. García-Gonzalo[1], S. de Lamo-Castellví[2]
[1]University of Zaragoza, Producción Animal y Ciencia de los Alimentos, Zaragoza, Spain
[2]Universitat Rovira i Virgili, Departament d'Enginyeria Quimica- Food- Innovation & Engineering, Tarragona, Spain

Backgrounds

Fourier transform infrared (FT-IR) and Raman (FT-Raman) spectroscopy combined with multivariate analysis can be used for detection, classification, and identification of food-related microorganisms because FT-IR and FT-Raman spectra provide bands from all their cellular components.

Objectives

The main objectives of the present research were to study the potential of these techniques combined with soft independent modeling of class analogy multivariate analysis to discriminate and detect biochemical differences of *Escherichia coli* MG1655 wild-type (WT) strain and its genetic stress-resistant variants CAR, CIT and LIM [mutants selected by subculturing with the antimicrobials carvacrol, citral and (+)-limonene oxide, respectively].

Methods

WT and variants were grown at 37°C for 24h. Then, bacterial cultures were centrifuged and washed twice with 0.1% Peptone Water. For IR analysis, pellets were placed onto ATR diamond crystal, vacuum dried and IR spectra were collected in the mid-infrared region (4000-800 cm^{-1}). For Raman experiments, pellets were placed onto a silicon film and Raman spectra were obtained with Argon laser at 514 nm with power of 25 mW (950-1800 cm^{-1}). IR and Raman data were analysed by soft independent modeling of class analogy (SIMCA) building up 2-classes SIMCA models with IR and Raman data from WT and variants CAR, CIT and LIM.

Conclusions

Bacterial components producing their major discrimination were lipids per CAR, phospholipids per CIT and carbohydrates or peptidoglycan per LIM. This study has shown the potential of FT-IR and FT-Raman combined with SIMCA to discriminate *E. coli* variants and detect biochemical differences among the WT and stress-resistant mutants.

FEMS7-2111
Food Microbiology

INCREASED RESISTANCE TO ANTIBIOTICS OF GENETIC VARIANTS OF ESCHERICHIA COLI MG1655 ISOLATED BY INDIVIDUAL CONSTITUENTS OF ESSENTIAL OILS

D. Berdejo[1], *D. García-Gonzalo*[1], *R. Pagán*[1]
[1]*University of Zaragoza, Producción Animal y Ciencia de los Alimentos, Zaragoza, Spain*

Backgrounds

Presence of stable stress-resistance microbes can compromise food safety. Several stresses applied during food preservation treatments, such as heat, acid or high pressure, have been shown to generate genetic stress-resistance variants derived from contaminating microorganisms. In a previous research, we isolated the genetic variants CAR, CIT and LIM after continuous growth of wild-type (WT) *Escherichia coli* MG1655 in presence of sub-inhibitory concentrations of the natural antimicrobials: carvacrol, citral or (+)-limonene oxide, respectively. These mutant strains showed an increased resistance against these antimicrobials. Study of cross-resistance against other antimicrobials, such as antibiotics, would provide information related to bacterial mechanisms of inactivation/resistance against the tested antimicrobials.

Objectives

The main objective of this research was to compare the antibiotic resistance of WT with that of CAR, CIT and LIM mutants.

Methods

The agar disk diffusion assay was used to test the susceptibility of mutant and WT strains to the following antibiotics: ampicillin, trimethoprim, chloramphenicol, tetracycline, rifampicin, kanamycin, novobiocin, norfloxacin, cephalexin and nalidixic acid. Impregnated disks were placed on the surface of inoculated plates with mutants and WT. After incubation of plates at 37ºC for 24h, diameters of the resulting inhibition zones were measured.

Conclusions

The three mutants showed smaller zones of growth inhibition for all the tested antibiotics, except for rifampicin, than WT (p<0.001). The inhibition halos for the CIT strain caused by ampicillin, chloramphenicol and novobiocin were reduced more than 50%. The development of cross-resistance against antibiotics would suggest similar mechanisms of inactivation and/or resistance against the tested natural antimicrobials and antibiotics.

FEMS7-0057
Food Microbiology

EFFECTS OF GAMMA IRRADIATION ON MICROBIAL AND SENSORY PROPERTIES AND SHELF LIFE OF IRANIAN NATIVE FRESH BARBERRY FRUITS

S. Berenji Ardestani[1], H.R. Akhavan[2]
[1]NSTRI, Research School of Radiation Applications, Tehran, Iran
[2]Shahid Bahonar University of Kerman, Department of Food Science and Technology, Kerman, Iran

Backgrounds

Berberis vulgaris is one of the most important Iranian native fruit with the produce about 16539 tones in 2014. The storage time of fresh fruits is short because of physiological maturity, biochemical changes and ethylene produce. At refrigerator temperature in less than 10 days they will lose their color, texture firmness, flavor and gradually, they will become acidified and spoiled, microbiologically. Gamma irradiation is able to enhance the safety and shelf life of fresh fruits by inhibiting microbial growth.

Objectives

Gamma irradiation has been shown to greatly reduce potential microbiological risk of fresh fruits, resulting in improved microbial safety as well as extending their shelf life. In this study the effects of gamma irradiation at doses of 0.5-2 kGy on spoilage, microbial and sensory properties of fresh barberry (*Berberis vulgaris*) fruits during 40 days refrigerated storage were studied.

MethodsThe percentage of fruits decay, microbial evaluations including fungi total counts (log cfu gr^{-1}) and sensory properties such as color, flavor and taste, aroma and odor, texture and total acceptance by means of 9 points hedonic test by 12 panelists, were studied.**Conclusions**The decay and spoilage of control and irradiated samples at doses of more than 1.25 kGy were different, significantly and they were 44% and 10%, respectively. Irradiation, especially at the doses above 1.5 kGy caused microbial growth inhibition during 40 days storage, completely.
Generally, with respect to gamma irradiation effects on physicochemical, microbial, sensory characteristics and total acceptance by consumers, dose range of 1.25-2 kGy to increase fresh fruits shelf life was optimum and suggested.

FEMS7-0281
Food Microbiology

GENOME SHUFFLING OF LACTOBACILLUS PLANTARUM FOR THE ENHANCEMENT OF PRODUCTION OF BACTERIOCIN

S. Bhutada[1]
[1]*Sanjivani Arts- Commerce and Science College- Kopargaon, Department of Microbiology, Ahmednagar, India*

Backgrounds

Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria. The application of bacteriocins as food preservatives could be achieved either by using the bacteriocinogenic strain as a starter culture or by using the bacteriocin as a food additive.

Objectives

Genome shuffling is an important method for rapid improvement in microbial strains for desired phenotype. The main objectives of this study were to improve the bacteriocin production from *Lactobacillus rhamnosus* through genome shuffling.

Methods

In the present study, a total of 20 samples, namely unpasteurized milk (5), curd (5), butter (5), and cheese (5) was collected from a local dairy farm. The nine Lactic Acid Bacteria were isolated and screened from 20 different samples. LABs were identified on the basis of standard morphological, biochemical, physiological characteristics. The five mutagens were used; UV radiations, pH variation, temperature variation, Acridine orange and Ethidium bromide. The mutants were selected and tested for their bacteriocin production ability by assay after production and purification by salting out and dialysis, and antibacterial activity was examined. The UV mutation has improved isolated strain of *Lactobacillus plantarum* to enhance the production of bacteriocin to higher level than others. Hence, UV mutation was considered as an effective mutation agent for strain improvement of bacteriocin producer.

Conclusions

The results demonstrated that the genome shuffling has been successful in engineering *L. plantarum*. In the future, this technology is a promising candidate to accelerate poorly characterized strains for enhancement of production of bacteriocin

FEMS7-2478
Food Microbiology

BACTERIAL DIVERSITY OF TRADITIONAL SOURDOUGH MADE FROM THE WHEAT, SPELT AND RYE WHOLEMEAL FLOUR DETERMINED BY CULTURE-INDEPENDENT APPROACH

J. Boreczek[1], J. Gawor[2], D. Izak[1], J. Bardowski[1], R. Gromadka[2], M. Kowalczyk[1]
[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Department of Microbial Biochemistry, Warsaw, Poland
[2]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Laboratory of DNA Sequencing and Oligonucleotide Synthesis, Warsaw, Poland

Backgrounds

The use of the sourdough for traditional bread making is one of the oldest biotechnological processes in cereal food production. Sourdough bread is prepared from a mixture of flour and water that is fermented with lactic acid bacteria (LAB). Spontaneously fermented sourdoughs contain a lot of diverse microorganisms, especially LAB.

Objectives

The aim of this study was to identify bacterial diversity and understanding of microbiota changes of the spontaneous sourdoughs made from the wheat, spelt or rye wholemeal flour during 96h fermentation.

Methods

Sourdoughs samples were analysed by culture-independent technique. Next-Generation Sequencing of the V3 and V4 variable regions of the 16S rRNA genes was performed using the Illumina sequencing technology. DNA sequencing data were analysed with MOTHUR software based on the Silva reference database. The taxonomical composition was presented on the Krona Charts and the structures of the communities of all samples were compared using the Yue and Clayton measure.

Conclusions

In conclusion, it is the first attempt to determine microbial biodiversity of sourdoughs made from the wheat, spelt or rye wholemeal flour. Culture independent analyses show how the microbial ecology changes during 96h fermentation. *Weissella* genus dominated after 24h fermentation of the rye sourdough, but as the fermentation progressed, its abundance decreased in favor of the *Lactobacillus* genus. *Lactobacillus* genus was dominant in all sourdoughs after 96h, which is in accordance with our previous results obtained by culture-dependent analyses (data not shown).

This work was supported by the Applied Research Programme of the National Centre for Research and Development. PBS2/B8/12/2014 (FunCHLEB).

FEMS7-1106
Food Microbiology

STUDY OF ANTIMICROBIAL EFFECT OF STEVIA REBAUDIANA EXTRACTS ON ESCHERICHIA COLI GROWTH

S. Botella[1], *A. Boukharouba*[1], *M.L. Castelló*[2], *E. Doménech*[2]
[1]*Universitat Politècnica de València, Biotechnology, Valencia, Spain*
[2]*Universitat Politècnica de València, Institute of Food Engineering, Valencia, Spain*

Backgrounds

Stevia rebaudiana is a natural alternative to artificial sweetener, which can be used as substitutes for sucrose. In Europe rebaudioside A is accepted as a food additive since 2011. Furthermore, there is an increasing interest in the use of natural ingredients able to avoid the proliferation of microorganisms. In this framework, the potential use of *Stevia* as a natural preservative has recently been studied by various researchers.

Objectives

The present work evaluates the antimicrobial effect of *Stevia* on *E. coli.*

Methods

Two different extracts of *Stevia* were tested by obtaining growth curves of *E. coli*: a marketed purified extract from leaves of *Stevia* which mainly contain steviol glycosides with a high percentage of rebaudioside (98%), and an extract prepared from dried leaves at 2.5 % of concentration. The enumeration of *E. coli* was performed with a modified enumeration method following UNE-EN/ISO 4833:2003.

Conclusions

An antimicrobial effect was observed with the leaf extract in relation to control. However non effect was found with the commercial purified extract under the conditions studied. The leaf extract of *Stevia* is not only a sweetener but also introduces a preservative effect against *E. coli*.

Acknowledge

The authors would like to thank the Generalitat Valenciana, for funding the project GV/2013/029.

FEMS7-1151
Food Microbiology

EFFECT OF ERYTHROMYCIN IN GOAT'S MILK ON PROTEOLYSIS AND LIPOLYSIS OF ARTISANAL MATURED CHEESE

S. Botella[1], P. Quintanilla[2], L.V. Ambrosio[2], M.C. Beltrán[2], M.P. Molina[2]
[1]Universitat Politècnica de València, Biotechnology, Valencia, Spain
[2]Universitat Politècnica de València, Institute of Animal Science and Technology ICTA, Valencia, Spain

Backgrounds

The presence of antibiotic residues in dairy products can be a problem for public health because they cause allergies, digestive disorders and development of resistance, and also for the dairy industry as the cause of technical problems and product quality especially in fermented products as cheeses. Information on the effect of the presence of antibiotics in milk on the characteristics of the cheese is very limited.

Objectives

The objective of this study was to analyze the effect of the presence of erythromycin in goat's milk on the proteolysis and lipolysis characteristics and their related microorganism groups of matured cheese.

Methods

The elaborations of cheese were made, in triplicate, from raw goat milk without antibiotic and with addition of erythromycin to a concentration of 40 µg/kg (Maximum Residue Limit-MRL). The proteolysis and lipolysis levels, the enumeration of proteolytic and lipolytic microorganisms and also the presence of the residual antibiotic by HPLC were determined in cheeses, ripening at different times (1, 30 and 60 days).

Conclusions

The presence of erythromycin caused no differences in any of the parameters studied. Instead the period of ripening affects to all parameters, those values more high for the proteolysis, lipolysis and enumeration of proteolytic microorganisms and them more low of lipolytic counts is observed for the cheeses of 60 days of maturation. The content of antibiotic in the cheese was always lower than the MRL established for erythromycin in milk.

Acknowledge

The authors would like to thank the Ministerio de Ciencia e Innovación (Spain), for funding the project AGL2013-45147-R.

FEMS7-2244
Food Microbiology

EFFECT OF LACTOBACILLUS FERMENTUM ON THE ASSOCIATION OF CRONOBACTER SAKAZAKII TO CACO-2 CELLS

D. Brady[1], *C. Kealey*[1]
[1]*Athlone Institute of Technology, Life & Physical Science, Athlone, Ireland*

Backgrounds

Cronobacter sakazakii is an emerging opportunistic pathogen most often associated with the feeding of powdered infant formula (PIF). It has been suggested that breast-fed infants develop a gut microflora richer in *Lactobacilli* and *Bifidobacteria* with reduced levels of pathogenic bacteria when compared to formula-fed infants. *Lactobacilli* have been shown to have many beneficial functions in terms of protecting the host from gastrointestinal pathogens. Recently, a species of *Lactobacillus* has also been isolated from whey protein concentrate (WPC). WPC is a common ingredient in PIF and has been attributed with anti-adhesion and anti-invasion properties against *Cronobacter* spp.

Objectives

The present study aims to investigate the effects of *Lactobacilli sp* on the adhesion and invasion of *C.sakazakii* (ATCC® 29544) in relation to differentiated monolayers of human enterocyte-like cells.

Methods

Association assays were conducted with test material (live or heat killed organisms) added at the same time as the pathogenic organism *C.sakazakii* (ATCC 29544). Differentiated cell monolayers were exposed to the test materials 30 minutes prior to the addition of pathogenic species (*C.sakazakii* ATCC 29544).

Conclusions

A reduction in association was observed for live and heat killed *L.fermentum* (DSM 20052) and live SWPC bacterial isolate when compared to the negative control ($P < 0.01$). Heat killed SWPC bacterial isolate also reduced association ($P < 0.05$) but to a lesser degree than the live organism ($P < 0.05$).

FEMS7-2276
Food Microbiology

EFFECT OF ANTIMICROBIAL LIPIDS AND HUMAN SALIVA ON ORAL PATHOGENS

C. Creavan[1], N. Tumulty-Breslin[1], C. Redmond[1], C. Kealey[1], D. Brady[1]
[1]Athlone Institute of Technology, Life & Physical Science, Athlone, Ireland

Backgrounds

Streptococcus mutans is the primary etiological agent in the formation of human dental caries which is considered a major oral health problem in most industrialised countries. Multiple virulence factors allow the bacterium to successfully colonise the tooth surface and propagate becoming a numerically significant species within dental plaque. Other oral microorganisms assemble in plaque as a polymicrobial biofilm and *Porphyromonas gingivalis*, an important secondary colonizer in oral biofilms, has been implicated in periodontal disease.

Objectives

This study evaluated the *in vitro* antibacterial activity of food grade lipids and their respective fatty acid constituents on both *S. mutans and P. gingivalis growth and biofilm formation*. Micro-broth dilution assays were carried out to assess the viability of the oral pathogen in the presence of these food constituents and also in the presence and absence of saliva.

Methods

S. mutans was cultured from frozen stock on Brain Heart Infusion (BHI) agar supplemented with 5% defibrinated horse blood and grown aerobically for 48 h at 37°C. A single colony was then used to inoculate 20ml BHI broth and grown aerobically for a further 24 h at 37°C. To evaluate biofilm formation by *S mutans* the method described previously by Tamura et al., (2009) was employed. *P. gingivalis* was cultured in TSB supplemented with vitamin K1 and hemin and incubated at 37ºC in an anaerobic chamber.

Conclusions

Bacterial growth was not affected by any of the oils tested. A selection of MCFAs and LCFAs comprising capric, lauric, myristic, oleic and linoleic acid showed varying levels of bacteriocidal and bacteriostatic activity towards *S. mutans and P. gingivalis*. Interestingly saliva interfered with the bacteriocidal activity of the fatty acids *in vitro*.

FEMS7-0465
Food Microbiology

CONTROL OF FUNGAL SPOILAGE IN FEED

P. Bruinenberg[1], J. Dijksterhuis[2]
[1]Trouw Nutrition, R&D, Amersfoort, The Netherlands
[2]CBS-KNAW Biodiversity Centre, Applied and Industrial Mycology, Utrecht, The Netherlands

Backgrounds

Feed safety is a major issue throughout the world. Therefore, much attention is paid to control fungal spoilage of feed and the risk of mycotoxin production, which has adverse effects on farm animals.

Objectives

This study aims to isolate and identify major fungal species on poultry feed samples and to determine the efficacy and mode of action of selected inhibitors of fungal growth.

Methods

Fungal strains were isolated from contaminated poultry feed samples and typed by morphological appearance on feed and agar plates including microscopic evaluation. Furthermore, DNA sequencing of the ß-tubilin and calmodulin gene was applied to identify the fungal species. The minimally inhibitory concentration (MIC) of inhibitors of fungal growth was determined by incubation of spore suspensions in a microtiter plate format. The mode of action of inhibitors was assessed by measuring differences in membrane permeability of germinating conidia using the fluorescent probe TOTO-1 as a viability marker.

Conclusions

Dominant fungi isolated from poultry feed samples belong to the genus *Aspergillus* with an *Eurotium* sexual stadium: i.e. *A. chevalieri* and *A. proliferants*. In addition, *Fusarium equiseti, Penicillium lanosocoeruleum* and *Wallemia sebi* were identified. The *Aspergillus* species and *W. sebi* are known as xerophylic fungi: e.g. they can grow at dry conditions with aw < 0.8. Evaluation of the results of the MIC data set combined with (fluorescent) microscopy of conidia treated with various inhibitors provide insight in understanding their mode of action. The results of this study enable the development of effective solutions to prevent fungal spoilage of feed products.

FEMS7-3123
Food Microbiology

TRANS-EUROPEAN STUDY ON THE IMPLEMENTATION OF NOVEL WATER TESTING METHODOLOGIES TO ASSESS THE SAFETY OF IRRIGATION WATER

C. Burgess[1], B. Moreira[1], R. O'Malley[1], K. Richards[2], R. Santos[3], S. Monteiro[3], A. Rajkovic[4], N. Smigic[4], L. Avery[5], E. Pagaling[5], S. Pedley[6], A. Lopez-Aviles[6], M. Byrne[7], A. Martinez-Murcia[8]
[1]Teagasc Food Research Centre, Department of Food Safety, Dublin 15, Ireland
[2]Teagasc Johnstown Castle, Environmental Research Centre, Wexford, Ireland
[3]Instituto Superior Técnico, Laboratorio de Analises, Lisbon, Portugal
[4]University of Belgrade, Department of Food Safety and Quality Management, Belgrade, Serbia
[5]James Hutton Institute, Environmental and Biochemical Sciences, Aberdeen, United Kingdom
[6]University of Surrey, Department of Civil and Environmental Engineering, Guilford, United Kingdom
[7]City Analysts Ltd, Water Analysis Testing, Dublin, Ireland
[8]Genetic PCR Solutions, and University Miguel Hernández, Alicante, Spain

Backgrounds

Microbial contamination of fresh produce is a major health concern, and the number of produce associated disease outbreaks has increased in recent years. The development of user-friendly, validated, pathogen detection methods are of key importance for detecting and preventing microbial disease outbreaks and providing enhanced product assurance.

Objectives

The aim of this study is to demonstrate the utility of novel methods developed as part of a European project, Aquavalens, to determine the microbiological quality of water used in the food production, with a particular focus on irrigation water and processing water.

Methods

This study utilised Aquavalens developed methods for the detection of pathogens in water used in the production and/or processing of salad leaves, soft fruits, including raspberries and strawberries and sprouted seeds. Sampling sites were selected in Ireland, Portugal, Serbia and the United Kingdom. Water samples are analysed by qPCR for the presence of *Salmonella*, *E. coli* O157, Hepatitis A and Norovirus and the samples are tested in parallel for the microbiological parameters outlined in the European Union Drinking Water regulations.

Conclusions

A detailed sampling plan and standard operating procedure enabled the successful implementation of these methodologies across Europe. Harmonisation of methodologies enables the direct correlation of results and a robust testing platform for the assays. Advantages include that the method can save time and money because the bacterial, protozoan and viral pathogens are concentrated from water using a single filter and that PCR techniques are quicker than the traditional methods for detecting pathogens.

FEMS7-0092
Food Microbiology

ANTI-ADHESIVE AND ANTI-BIOFILM ACTIVITY OF ESSENTIAL OILS RICH IN CARVACROL AND THYMOL AGAINST SALMONELLA ENTERITIDIS

I. Cabarkapa[1], R. Colovic[1], O. Djuragic[1], B. Kokic[1], S. Popovic[1], Z. Tomicic[1]
[1]Institute of Food Technology,
RESEARCH CENTER FOR FEED TECHNOLOGY AND ANIMAL PRODUCTS, Novi Sad, Serbia

Backgrounds

Biofilms formed in food processing environments may represent a long-term source of foodstuff contamination. Given that bacteria enclosed into biofilm showed high tolerance/resistance to standard sanitizers, a great number of studies have been focused on evaluation of plant-based compounds as potential sources of antimicrobial agents, as they are effective, safe and eco-friendly.

Objectives

Aim of the present study was to determine the bioactive compounds in four essential oils (EO's) from *Origanum heracleoticum*, *Origanum vulgare*, *Thymus vulgaris* and *Thymus serpyllum* and to assess their antimicrobial, anti-adhesive and antibiofilm activity against *Salmonella* Enteritidis.

Methods

Strains were previously characterized depending on the expression of extracellular matrix components as an rdar (cellulose + curly fimbriae) and bdar morphotype (curly fimbriae). Antiadhesion tests were carried out by crystal violet and metabolic activity assay.

Conclusions

Results indicated that percentage of carvacrol and thymol in EO's of *O. heracleoticum, O. vulgare, Th. vulgaris,* and *Th. serpyllum* (71.60%, 63.60%, 59.77% and 40.04%, respectively) were proportional to their antimicrobial potencies. Comparing the efficacy of EO's to inhibit the initial adhesion and metabolic activity of cells between the strains of rdar and bdar morphotype, statistically significant differences were not observed. EO's showed weaker effect on the preformed biofilms. Results related to the effectiveness of EO's on the total biomass of biofilm and metabolic activity of the cells enclosed into biofilm, indicated that applied EO's caused a reduction in a dose-dependent manner over time. Additionally, in assessing efficacy of EO's in the treatment of preformed biofilms by isolates with expressed rdar morphotype compared to strains with expressed bdar morphotype, statistically significant differences were observed. It is assumed that this is a consequence of the presence of a more complex biofilm matrix that consists of cellulose and curly fimbriae in relation to the biofilm matrix of bdar morphotype isolates that consists of only curly fimbriae.

FEMS7-1635
Food Microbiology

CO-TRANSFER OF AMINOGLYCOSIDE, TETRACYCLINE AND MACROLIDE RESISTANCE GENES BETWEEN ENTEROCOCCUS STRAINS ISOLATED FROM READY-TO-EAT FOOD

W. Chajęcka-Wierzchowska[1], *A. Zadernowska*[1], *Ł. Łaniewska-Trokenheim*[1]
[1]*University of Warmia and Mazury in Olsztyn, Chair of Industrial and Food Microbiology, Olsztyn, Poland*

Backgrounds

Enterococcus spp. are one of the most common groups of bacteria in foods. Antibiotic resistance has been acquired, and has disseminated throughout enterococci, via horizontal transfer of mobile genetic elements. This transmission has been mediated mainly by conjugative plasmids of the pheromone-responsive.

Objectives

The purpose of this study was to conjugal transfer of resistance genes between a multi-resistant *E. faecium, E. faecalis* and *E. casseliflavus* isolates from ready-to-eat food and a sensitive *E. faecalis* strain.

Methods

Donor strains had tetracycline resistance genes (*tet* M, *tet*L, *tet*K, *tet*O), macrolides genes (*erm*A, *erm*B, *msr*C *mef*A/E) and/or aminoglycoside genes *(aac*(6 ')-*aph*(2' ') Ia, *aph* (3')-IIIa, *ant* (6 ')-Ia). The recipient was a reference strain of *E. faecalis* JH2-2 (LMG 19456) free of plasmids resistant to rifampicin and fusidic acid.

Conclusions

Among the tested strains isolated from food more than 70% were able to transfer by conjugation at least one of the held antibiotic resistance genes. The frequency of gene transfer was dependent on the strain and the transferred gene and was in the range from $1{,}2x10^{-6}$ to $3{,}1x10^{-8}$ transkonjugants per donor. Genes *tet*M, *erm*B and *aac*(6')-*aph*(2'') were transferred with the greatest frequency. Simultaneously with the *tet*M gene, *int* gene was transferred, indicating the participation in the conjugation process of the Tn*916*/Tn*1545* transposon family.
The results of the study indicated that ready-to-eat food can be an important vector in transmitting resistance to antimicrobial agents. This research was financed by the National Science Centre allocated on the basis of a decision number DEC-2013/09/N/NZ9/01630.

FEMS7-2526
Food Microbiology

ENTEROTOXIGENIC POTENTIAL, VIRULENCE FACTORS AND ANTIMICROBIAL RESISTANCE OF S. EPIDERMIDIS STRAINS ISOLATED FROM READY-TO-EAT FOOD

W. Chajęcka-Wierzchowska[1], *A. Zadernowska*[1], *Ł. Łaniewska-Trokenheim*[1]
[1]*University of Warmia and Mazury in Olsztyn, Chair of Industrial and Food Microbiology, Olsztyn, Poland*

Backgrounds

Coagulase-negative staphylococci (CoNS) are among the most widespread microorganisms in nature. For many years they were classified as microflora unable to cause disease. A consistent increase in the role of CoNS (especially *S. epidermidis*) in causing hospital infections has been observed in recent years.

Objectives

The aim of this work was to study the enterotoxigenic potential, virulence factors and phenotypic and genotypic antimicrobial resistance profile of *S. epidermidis* isolated from ready-to-eat food of animal origin.

Methods

Isolates were tested for resistance to 15 antibiotics by the disk diffusion method according to CLSI. PCR was used for the detection of antibiotic resistance genes encoding: methicillin resistance *mec*A; macrolide resistance; *erm*A, *erm*B, *erm*C, *mrs*A/B; efflux proteins *tet*K and *tet*L and ribosomal protection proteins *tet*M. The genes encoding enterotoxins as well as ACME element, *ica*ADBC operon and insertion sequence element IS256 were also investigated.

Conclusions

Almost 25% of staphylococcal isolates were multidrug resistant. Most of them were resistant to cefoxitin, clindamycin, rifampicin and erythromycin. All methicillin resistant staphylococci harboured *mec*A gene. Isolates, phenotypic resistant to tetracycline, harboured at least one tetracycline resistance determinant on which *tet*(M) was most frequent. In the erythromycin resistant isolates, the macrolide resistance genes *erm*C or *msr*A/B were present. *S. epidermidis* strains were also able to form biofilm. Over a half of them harbored ACME element, known to promote microbial growth and survival in infected organism. Additionally, 36% of strains harboured *ica*ADBC operon, and almost 15% of them possessed IS256.

This research was supported by grant Ministry of Science and Higher Education, Poland N N312 236138 and by UWM young research grant.

FEMS7-3266
Food Microbiology

IN VITRO EVALUATION OF ANTI¬¬-LISTERIA ACTIVITY OF TWO LACTIC ACID BACTERIA ISOLATED FROM DONKEY MILK

I. Chemsi[1], *B. Ali*[1], *E. Bouchra*[1], *S. Abdelaziz*[1]
[1]*Faculty of Sciences, Biology, Casablanca, Morocco*

Backgrounds

The interest of scientists for natural molecules with an antimicrobial effect has continued to increase. With a view to reducing the use of synthetic food additives, research and study of natural antibiotics such as bacteriocins is gaining more attention. Bacteriocins constitute a set of proteins produced by lactic acid bacteria (LAB) and possessing antagonistic activity directed against pathogenic bacteria colonizing the food products.

Objectives

The aim of the study is to identify some LAB that produce bacteriocins directed against one of the most common pathogenic bacteria: *Listeria monocytogenes.*

Methods

The isolation of the LAB was carried out on M17 and MRS medium from donkey milk. The preliminary identification is effectuated with conventional microbiological method and biochemical (Gallery API20E) processes. Genotypic identification of the LAB strains is carried out by DNA fragments amplification then the resulting DNA is sequenced. The sequences are analized with bioinformatics tools. After optimization of the bacteriocin secretion, the antagonistic activity of LAB effect was tested through the well diffusion assay. The indicator strains used is the pathogenic bacterium *Listeria monocytogenes.*

Conclusions

Among the isolated and identified bacteria, Leuconostoc mesenteroidess and Staphylococcus succinus showed major antimicrobial activity. This study highlights the important biotechnological potential of these bacteriocins that can be exploited in the agro-food industry (bio-preservatives) and pharmaceutical (therapeutic antibiotics).

FEMS7-1001
Food Microbiology

PREVALNECE OF FOODBORNE PATHOGENS IN SLAUGHTERED CHICKEN CARCASSES IN SOUTH KOREA

B. Choi[1], O. Jeong[1], C. Lim[1], S. Youn[1], B. Jeon[1], H. Lee[1], H. Lee[1], M. Kang[1]
[1]Animal and plant quarantine agency, Avian Disease Division, Gimcheon-si, Republic of Korea

Backgrounds

Backgrounds: Public health issues have gradually increased for decades especially focusing on foods including meat and meat products. Chicken meat and products are considered as a common source of foodborne diseases in humans. There are a variety of pathogenic bacteria contaminating chicken meat such as *Salmonella* spp., *Staphylococcus aureus*, *Clostridium* perfringens, *Campylobacter* spp., *Escherichia coli*, *Yersinia enterocolitica*, and *Listeria moncytogenes*.

Objectives

Objectives: This study was intended to evaluate the prevalence of pathogenic bacteria in chicken carcasses freshly slaughtered in South Korea.

Methods

Methods: Two hundred seventy samples of chicken carcasses were collected from 27 chicken slaughterhouses throughout the nation in South Korea during 2014 -2016. All samples was tested for presence of *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter* spp., pathogenic *Escherichia coli*, *Yersinia enterocolitica*, and *Listeria moncytogenes* according to the procedure of Korean Food Standard Codex for meat and meat products.

Conclusions

Conclusions: *Clostridium perfringens* (33.7%), *Staphylococcus aureus* (24.1%), and *Salmonella* spp. (17.4%) are frequently isolated from chicken carcasses tested in this study. The prevalence of other pathogenic bacteria were as follows: *Campylobacter coli* (15.6%), *Campylobacter jejuni* (10.4%), *Escherichia coli* O111 (3.6%), *Yersinia enterocolitica* (1.9%), *Escherichia coli* O145 (1.5%), O26 (1.1%), and *Listeria monocytogenes* (0.7%). This study indicates stronger safety systems are needed to decrease bacterial contaminations in chicken meat in South Korea.

FEMS7-2121
Food Microbiology

PROTECTION ACTIVITY OF A NOVEL PROBIOTIC STRAIN OF LACTOBACILLUS SP. AGAINST FOOD BORNE PATHOGEN INFECTION

H. Choi[1], H. Park[1], S. Lee[1], J. Park[1], D. Jo[1], S. Oh[1], S. Kim[1]
[1]National Institute of Crop ScienceNICS Rural Development AdministrationRDA, Crop post harvest technology, Suwon, Republic of Korea

Backgrounds

Lactobacillus enhances intestinal epithelial barrier function; they also compete with and suppress pathogenic bacteria, and modulate immune system activity. Thus, Lactobacillus has significant therapeutic potential to overcome intestinal disorders and can be used as usual functional food ingredients and biological preservatives. However, general mechanisms of action by which probiotic Lactobacilli exert their beneficial effects are still largely unknown.

Objectives

In this study, we investigated the effects of Lactobacillus sp. on the growth of S. Typhimurium.

Methods

We previously isolated Lactobacillus sp. from traditional Korean fermented soybean food, and exhibits strong enzymatic and antimicrobial activity against food-borne pathogens. We analyzed S. Typhimurium-induced cytotoxicity by stimulating the host immune response using tools with genetic and protein levels.

Conclusions

The results showed that when S. Typhimurium was co-incubated with Lactobacillus sp., the growth of S. Typhimurium was reduced, and the S. Typhimurium-induced NF-κB activation, Akt and p38 phosphorylation were decreased. This study indicates that Lactobacillus sp. has the potential probiotic properties, and exhibits strong inhibition activity against S. Typhimurium infection. Our findings also could be used as therapeutic and preventative agents, to control of pathogenic diseases.

FEMS7-3017
Food Microbiology

PRESENCE OF NOROVIRUS AND HEPATITIS A IN RASPBERRIES

R. Čobanović[1], *K. Mihajlović*[1]
[1]*SP Laboratory, Microbiological, Bečej, Serbia*

Backgrounds

Serbia is the largest exporter of raspberry in EU. Raspberry is a perishable food which can be consumed as fresh or minimally-processed as well as a frozen ingredient added to many foods. According to EFSA, contamination by Noroviruses and Hepatitis A viruses may occure at various stages of berry production chain.

Objectives

Based on above mentioned, the aim of this study was to evaluate virological safety of raspberry grown in Serbia.

Methods

Analyzed samples were collected from various independent producers from January 2014 untill December 2016. Samples were analyzed on Norovirus (NoV) genogroups I (GI) and II (GII) - 545 samples and Hepatitis A (HAV) - 418 samples. The applied method was based on ISO/TS 15216-2:2013 Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -- Part 2: Method for qualitative detection. Out of 963 analyzed samples neither one showed unsatisfactory positive result concerning both NoV genogroups I (GI) and II (GII) and HAV viruses.

Conclusions

As far as obtained results are concerned it can be concluded that Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) are properly implemented throughout raspberry production chain in Serbia. Due to high risk of contamination during raspberry production it is necessary to conduct permanent monitoring in order to provide microbiologicaly safe products that are exported to the EU.

FEMS7-1942
Food Microbiology

MINING MICROBES FROM DAIRY PRODUCTS AND ASSOCIATED PROCESSING ENVIRONMENTS FOR THE IDENTIFICATION OF NOVEL INHIBITORS OF QUORUM SENSING AND BIOFILM FORMATION

L. Coughlan[1,2], P. Cotter[1,3], C. Hill[2,3], A. Alvarez-Ordóñez[4]
[1]Teagasc Food Research Centre, Food Biosciences, Cork, Ireland
[2]University College Cork, School of Microbiology, Cork, Ireland
[3]APC Microbiome Institute, Microbiology, Cork, Ireland
[4]University of León,
Department of Food Hygiene and Technology and Institute of Food Science and Technology, León, Spain

Backgrounds

Coordinated by the quorum sensing (QS) gene regulatory system, biofilm formation can allow spoilage and pathogenic bacteria to persist in the dairy industry, impacting food safety and quality.

Objectives

This study focused on mining microbes from dairy products and associated environments for the identification of bacterial species with QS inhibitory activity for further evaluation as biofilm inhibitors.

Methods

Culturable bacterial collections comprising ~3,000 strains were constructed from 12 samples of milk, cheese and dairy processing environments after plating of serial dilutions on brain heart infusion (BHI) agar and de Man, Rogosa and Sharpe (MRS) agar and incubation for 48h under anaerobic (for BHI) or anaerobic (for MRS) conditions at 5, 37 and 55°C. Strains retrieved (up to ten of each morphology per sample) underwent an initial screening process in which isolates displaying QS inhibitory (QSI) activity were readily detected using overlay assays employing *Chromobacterium violaceum* (auto-inducer 1 QS) and *Vibrio harveyi* (auto-inducer 2) as indicator organisms. All strains presumptively identified as QSIs were identified through 16S rRNA gene sequencing and are currently being characterized for their ability to inhibit biofilms of various common spoilage and pathogenic microorganisms (*Pseudomonas* spp., *Listeria monocytogenes*, *Cronobacter sakazakii*, *Geobacillus stearothermophilus*).

Conclusions

Sixteen confirmed AI-1 QSIs were identified as *Hafnia alvei* and 54 AI-2 QSIs as *Lactococcus lactis* and *Lactobacillus plantarum*. Current work assesses the ability of these strains to inhibit biofilms of selected common spoilage and pathogenic microorganisms. Future study will include examining their efficacy in preventing biofilm formation in dynamic biofilm models and industrial settings.

FEMS7-2401
Food Microbiology

GENETIC TYPING AND TECHNOLOGICAL EVALUATION OF SACCHAROMYCES ISOLATES COLLECTED FROM BRAZILIAN VINEYARDS

G. Crosato[1], C. Nadai[1], M. Carlot[1], J. Garavaglia[2], L. Massochin Nunes Pinto[2], D. Righetto Ziegler[2], R.C. de Souza Ramos[2], R. Cassanta Rossi[2], A. Giacomini[1,3], V. Corich[1,3]
[1]Università degli Studi di Padova,
Dipartimento di Agronomia Animali Alimenti Risorse naturali e Ambiente - DAFNAE, Legnaro, Italy
[2]Universidade do Vale do Rio dos Sinos UNISINOS,
Instituto Tecnológico em Alimentos para a Saúde itt Nutrifor, São Leopoldo RS, Brazil
[3]Università degli Studi di Padova,
Centro Interdipartimentale per la Ricerca in Viticoltura ed Enologia C.I.R.V.E., Conegliano, Italy

Backgrounds

Saccharomyces is a worldwide spread yeast genus. It includes the most important fermenting agent in wine industries, *Saccharomyces cerevisiae* and it is adapted to several environments. The use of autochthonous *Saccharomyces* strains in the winemaking process can emphasize the wine *terroir*. Compared to Europe, South of Brazil has high oenological potential. Besides the climate, the main difference between the two areas is the winemaking and vine growing history: ancient for the former and recent for the latter.

Objectives

We investigated the characteristics of a group of *Saccharomyces* strains collected in "Vale dos Vinhedos" vineyards (RS – Brazil) in order to find out the oenological properties of the local yeast population. We analyzed the genetic variability of the yeast isolates, some important genetic traits and their impact on yeast phenotypes, in order to better understand the adaptation of the population to the vineyard environment.

Methods

MtDNA RFLP analysis has been used to genotype the yeast population. Fermentations were run in synthetic must to obtain yeast fermentation performance. Sulfite tolerance under fermentation conditions was evaluated and copper resistance, as well.

Conclusions

The collected isolates, generally, showed good oenological properties, regarding both fermentation kinetics and sulfite/cupper tolerance. Thus, they can be further investigated to select strains able to improve the quality and the terroir of Brazilian wines.

FEMS7-2034
Food Microbiology

IMPACT OF PLANT STEROLS ON INTESTINAL MICROBIOTA FROM LEAN AND OBESE SUBJECTS USING TIM-2 IN VITRO MODEL

M. Cuevas Tena[1], C. Bussolo[2], A.D. Silva[3], A. Alegría[1], M.J. Lagarda[1], K. Venema[4]
[1]Universitat de Valencia,
Medicina Preventiva y Salud Pública- Ciencias de la Alimentación- Toxicología y Medicina Legal, Valencia, Spain
[2]University of Groningen, Department of Microbiology, Groningen, The Netherlands
[3]University of Sao Paulo, Department of Biochemical and Pharmaceutical Technology, Sao Paulo, Brazil
[4]Maastricht University-Campus Venlo, Healthy Eating and Food Innovation, Venlo, The Netherlands

Backgrounds

Plant sterols (Ps) are poorly absorbed in the small intestine (1-2%) (Quilez et al., 2003) reaching the colon where they are biotransformed by intestinal microbiota. Some authors have studied this biotransformation (Rosenfeld et al., 1954; Snog-Kjaer & Dam, 1956; Eyssen et al., 1973 and 1974). Baumgartner et al, (2016) are the only ones who analyze the effect of microbiota from individuals after consumption of plant stanol ester enriched margarines (3.0g/day), and they have not observed significant changes in microbiota composition. Therefore, the effect of Ps on the microbiota remains unknown.

Objectives

To know the influence of Ps on the microbial population from lean and obese individuals using a validated *in vitro* dynamic fermentation system.

Methods

The TNO Intestinal Model 2 (TIM-2) was applied to a Ps standard and a Ps ingredient used in the manufacture of a fruit-based milk beverage. Changes of the colonic microbiota were evaluated by next generation sequencing. In addition, short chain fatty acids (SCFA), lactate and ammonia produced during fermentation were determined by ion exclusion chromatography (IEC*), Boehringer* UV-method and *Berthelot* reaction (spectrophotometry), respectively. For this purpose, the lumen and dialysate obtained after *in vitro* colonic fermentation at different times (0, 24, 48 and 72 hours) were analyzed.

Conclusions

Results will be available for presentation by the time of the congress.

FEMS7-3093
Food Microbiology

CHANGES OF BIOLOGICALLY ACTIVE COMPOUNDS IN APPLE POMACE UNDER THE INFLUENCE OF LACTIC ACID BACTERIA.

A. Czyżowska[1], *A. Wilkowska*[1], *I. Motyl*[1], *A. Nowak*[1]
[1]*Institute of Fermentation Technology and Microbiology,*
Department of Biotechnology and Food Sciences- Lodz University of Technology, Łódź, Poland

Backgrounds

Apple pomace is a waste product in the fruit industry. It may be a rich source of polyphenols such as quercetin glycosides, dihydrochalcones, cinnamic acid derivatives, flavan-3-ols. During the process it is subjected to enzymatic treatment in order to facilitate processing. One of the ways of using, is to add it as a prebiotic component.

Objectives

In this study, apple pomace from Cortland variety was used, and subjected to hydrolysis by preparations Cellulosoft and Viscozyme.

Methods

On the obtained hydrolyzates were cultivated lactic acid bacteria: *Lactobacillus rhamnosus* 900 and 908, *L. casei* 904, 919 and 906, and *L. paracasei* 915 and 920. Cultures were grown for 17 days at 37°C. Test samples were cultures for the days on which the microorganisms were in the final stage of the logarithmic phase or reached a stationary phase.

Conclusions

Gallic acid was reported in highest amounts from 5.269 mg/100ml (H/920) to 0.573 mg/100ml (H/919). Catechin and procyanidin B2 were identified in two samples (H/904 and H/906). In all the samples revealed the presence of procyanidin B1 and quercetin derivatives. Procyanidin B1 concentration ranged from 0.076 to 0.435 mg/100 ml. Among the derivatives of quercetin highest amounts of quercetin-glucoside and quercetin-rutinoside were found. Phloretin was found in five, and phloridzin in six samples. Chlorogenic acid was identified in seven, neochlorogenic in only four samples, its concentration was in the range from 0.066 to 0.360 mg/100ml. The highest concentration of identified substances was found in samples H/920 and H/915, while the lowest in the H/906.

This work was supported through Narodowe Centrum Badań i Rozwoju (NCBiR) grant GEKON nr Gekon2/05/267284/15/2015.

FEMS7-3098
Food Microbiology

ARONIA BEVERAGES FERMENTED BY LACTIC ACID BACTERIA (LAB).

A. Czyżowska[1], A. Żołek[1], I. Motyl[1], A. Nowak[1]
[1]Institute of Fermentation Technology and Microbiology,
Department of Biotechnology and Food Sciences- Lodz University of Technology, Łódź, Poland

Backgrounds

The experimental material were beverages reconstituted from chokeberry concentrate.

Objectives

The concentrate was diluted with water to a pH of about 4.6, followed by adding sucrose to obtain approximately 6°BLG. Beverages were divided into two groups, one of which was pasteurized at 121°C for 10 minutes, and were inoculated with lactic acid bacteria in order to conduct lactic acid fermentation.

LAB: *Lactobacillus rhamnosus* 908 and 900*, Lactobacillus casei* 919, *Lactobacillus paracasei* 920*, Lactobacillus plantarum* 861 and *Lactobacillus brevis* strains M18 and 0945 were used. Inoculated beverages were incubated at 30°C for 2 and 7 days.

Methods

In all the samples was tested lactic acid concentration as lactic acid fermentation indicator. In addition: citric, acetic and propionic acids and ethyl alcohol were determined. In samples were also examined concentration of polyphenols.

Conclusions

The control samples contained only citric acid, lactic acid concentration was the highest (0.25 g/100 ml) in samples inoculated with strain 0945, the lowest with 920 strain, wherein traces of lactic acid were determined. In pasteurized samples containing strains 0945, 919 and 861, lactic acid content after 7 days of fermentation increased in comparison with 2-day fermentation.

Among the anthocyanins five cyanidin glycosides: galactoside, glucoside, rutinoside, arabinoside and xyloside were found. Among the phenolic acids four: neochlorogenic, p-coumaroylquinic, chlorogenic and cryptochlorogenic has been detected. It was also found the presence of six quercetin derivatives: sophoroside, vicianoside, robinobioside, rutinoside, galactoside and glucoside.

The content of the above-mentioned compounds, varied depending on the method of treatment of the samples and the strain used.

FEMS7-3106
Food Microbiology

EFFECT OF NISIN-BASED ADDITIVES ON THE GROWTH RATE OF LISTERIA MONOCYTOGENES AND LACTIC ACID BACTERIA IN RTE MEAT PRODUCTS

A. de Benito[1], A. Roijals[2], S. Porta[1], J. García-Pina[2], J. Gonzalez[2], B. Ruiz[1]
[1]AINIA- technological centre, Bioassays, Valencia, Spain
[2]CHEMITAL Food Techniques, Calidad, Barcelona, Spain

Backgrounds

Ensuring food security can still be a challenge for food producers and authorities, as new safety issues are arising regarding the use of artificial additives, antimicrobial resistance and emmerging pathogens. There is a need for the development of novel and more natural additives that meet the consumers‘ needs and preferences. This work is aligned with the recent recommendation by the Codex Alimentarius to discuss the draft provision for the use of nisin in heat-treated processed comminuted meat, poultry, and game products at a maximum level of 25mg/Kg

Objectives

To assess the antimicrobial effect of new additives formulated with nisin in cooked meat products

Methods

The effect of newly developed nisin-based additives was assessed by using microorganisms representatives of this type of products: *Listeria monocytogenes* and lactic acid bacteria (LAB). Tests were done in a cooked meat product that was produced with the addition of different doses of the additives. Cooked meat products were sliced, inoculated, vacuum packed and stored for at least 45 days under refrigeration conditions. Analysis of samples to estimate the growth rate of *Listeria monocytogenes* and lactic acid bacteria was done at several time intervals

Conclusions

Results show an inhibitory effect on the growth of *Listeria monocytogenes* and lactic acid bacteria by the use of the new additives on the products from doses of 11,2 mg nisin/Kg meat product This effect was increased in combination with refrigerating temperatures and enables to provide a safety barrier against *Listeria monocytogenes* as well as increase the shelf life of the products

FEMS7-0885
Food Microbiology

OCHRATOXIN A PRODUCTION BY PENICILLIUM NORDICUM IN DRY-FERMENTED SAUSAGES FOLLOWING TWO RIPENING PROCESSES

J. Delgado[1], *B. Peromingo*[1], *F. Núñez*[1], *M.A. Asensio*[1], *M. Rodríguez*[1]
[1]*Research Institute of Meat and Meat products University of Extremadura, Hygiene and Food Safety, Cáceres, Spain*

Backgrounds

Dry-fermented sausages are highly appreciated products from the South of Europe. Bacteria, yeasts and molds contribute to the generation of the desired sensory qualities. However, some molds are able to produce mycotoxins on sausages. Ochratoxin A (OTA), which is mainly produced by *Penicillium nordicum* and *Penicillium verrucosum* in dry-cured meat products, is the main mycotoxin of concern. OTA production is influenced by environmental factors such as temperature and moisture. Dry-fermented sausages are typically processed at either *c.a.* 12 °C or at about 15-18 °C.

Objectives

The objective of this work was to evaluate OTA production on dry-fermented sausages at two ripening temperatures.

Methods

Raw sausages were inoculated with 10^3 spores/cm^2 of *P. nordicum* and fermented in separate ripening chambers set at 12 °C, 84% relative humidity (RH) and 15 °C, 86% RH, for 28 days. Mycotoxin extraction was based on QuEChERS method and analyzed by HPLC-FLD (Agilent Technologies).

Conclusions

Ripening at 12 °C led to 2.6 mg/kg OTA production in 14 days, whilst ripening at 15 °C led to 2.9 mg/kg OTA in 7 days. Therefore, even ripening at the lower temperature was not adequate to ensure safe sausage processing. Therefore, effective means to control OTA production on sausages are required. Strategies based on biocontrol agents, such as molds or yeasts endowed with antifungal activity, should be proposed.

Acknowledgements: Work funded by the Spanish Ministry of Economy and Competitiveness, Extremadura Government and FEDER (AGL2013-45729-P, GR15108).

FEMS7-1605
Food Microbiology

DEVELOPMENT AND OPTIMIZATION OF A UHPLC–MS/MS METHOD FOR DETECTION AND QUANTIFICATION OF CYCLOPIAZONIC ACID IN DRY-CURED HAM

B. Peromingo[1], J. Delgado[1], F. Núñez[1], P. Padilla[1], L. Sánchez-Montero[1], M. Rodríguez[1]
[1]Research Institute of Meat and Meat Products University of Extremadura, Hygiene and Food Safety, Cáceres, Spain

Backgrounds

Cyclopiazonic acid (CPA) is a mycotoxin produced by *Penicillium* and *Aspergillus* species that are able to grow on the surface of dry-cured meat products during the ripening process. CPA is a molecule difficult to detect because it can be degraded throughout chemical and physical processes.

Objectives

The objective of this study was to optimise an uHPLC–MS/MS method for detection and quantification of CPA in dry-cured ham-based matrices.

Methods

Four different extraction methods were evaluated. Mobile phases, flow rates, gradient mode and nature of extract solvent for optimising chromatographic parameters were tested. Results showed that for CPA isolation from cured matrices a QuEChERS-based method with some modifications was the most effective one. The extracts were redissolved in buffered ammonium acetate/methanol (30/70) before analysis by uHPLC-MS/MS. Regarding chromatographic parameters, the mobile phase contained 10 mM ammonium acetate (pH 5.75, acetic acid)/methanol. Elution was carried out in gradient mode and the flow rate was 0.2 mL/min. MS detection of CPA was performed using the precursor ion 335, and the quantitation ion 180. The run time was 15 min, being CPA detected at 5.6±1 min.

Conclusions

In conclusion the uHPLC-MS/MS method offers a useful, rapid and efficient tool for screening CPA in dry-cured meats. The early detection of CPA in these products would allow taking corrective actions to avoid risks associated with CPA contamination.

Acknowledgements: Funded by Spanish Ministry of Economy and Competitiveness, Government of Extremadura and FEDER (AGL2013-45729-P, GR15108). B. Peromingo and P. Padilla are recipient of a pre-doctoral fellowship (BES-2014-069484 and PEJ2014-P-0057 respectively).

FEMS7-3184
Food Microbiology

RAPID DETECTION OF MICROBIAL CONTAMINATION IN UHT MILK: PRACTICAL APPLICATION IN DAIRY INDUSTRY

K. Demnerova[1], M. Kracmarova[1], H. Stiborova[1]
[1]University of Chemistry and Technology Prague, Department of Biochemistry and Microbiology, Prague, Czech Republic

Backgrounds

The formation and impacts of biofilms on quality of milk in dairy industry is proven. Therefore, the contamination in manufacturing chain has to be identified quickly. Microbial quality of ultra-high temperature (UHT) milk is usually analysed by a traditional cultivation technique known as total bacterial count (TBC). However, this method is time demanding.

Objectives

Therefore, we tested and compared two faster methods based on different principles. The comparison was directed on: (i) the overall time required for the detection of bacterial spoilage; (ii) the length of pre-incubation time of UHT milk, which plays a key role in proliferating bacteria; (iii) simplicity of procedure and (iv) accuracy of results.

Methods

The first method, tested by two separate instruments from different producers, detects microbial contamination by measuring adenosine triphosphate (ATP) bioluminescence; the second, which is more novel and was tested by another instrument, detects it by analysing oxygen consumption. In the laboratory experiments, samples of UHT milk were inoculated with a low concentration of microorganisms (colony-forming unit per litter). These methods, together with TBC as a control, were used to measure microbial contamination in the samples. Then, all methods were trialled in a dairy processing plant.

Conclusions

While both methods determined microbial quality in a significantly shorter time, only one instrument satisfied all the requirements for operations in the dairy industry. Indeed, the dairy processing plant introduced this instrument into their microbial control process as an alternative to TBC.

Acknowledgement: The project was funded by the Czech Science Foundation projects no. 17-15936S.

FEMS7-2259
Food Microbiology

COLONISATION OF MEAT BY ESCHERICHIA COLI O157:H7: BACTERIAL TROPISM RESPECTIVE TO THE DIFFERENT TYPES OF SKELETAL MUSCLES, SUBTYPES OF MYOFIBERS AND POSTMORTEM TIME

M. Desvaux[1], C. Chagnot[2], A. Venien[3], S. Renier[2], N. Caccia[2], M. Refregiers[4], R. Talon[2], T. Astruc[3]
[1]INRA Auvergne-Rhône-Alpes, UMR MEDiS, Saint-Genès Champanelle, France
[2]INRA, UMR454 MEDIS, Clermont-Ferrand, France
[3]INRA, UR370 QuaPA, Clermont-Ferrand, France
[4]Synchrotron SOLEIL, Disco Beamline, Gif-sur-Yvette, France

Backgrounds

Escherichia coli O157:H7 are enterohaemorrhagic *E. coli* (EHEC) responsible for serious pediatric diseases and of great concern for the meat industry. Meat contamination by EHEC occurs at slaughtering, essentially at dehiding stage, where bacteria can be transferred from hides to carcasses. The skeletal muscle tissues are constituted of four major types of myofibers surrounded by the extracellular matrix (ECM).

Objectives

Despite the frequency of the associations between bacteria and meat, the molecular mechanisms of adhesion and the tropisms of bacteria across the different types of muscle fibers are totally unknown.

Methods

Bacterial adhesion to meat was investigated considering the different constituent myofibers of different skeletal muscles as well as postmortem evolution of muscle following fluorescence and electronic microscopy as well as immunohistochemical analyses.

Conclusions

Differential *E. coli* O157:H7 adhesion occurred at the surface of divergent skeletal muscle types. By analysing the adhesion of *E. coli* O157:H7 to model glycolytic and oxidative skeletal muscles, we provide the first in-depth interaction of EHEC with meat at cellular and tissue levels. While no significant differences could be observed for bacteria adhering to the different types of myofibers, bacterial adhesion essentially occurred at the ECM. Information on spatial localization of *E. coli* O157:H7 to meat was further provided and demonstrated their ability to adhere to skeletal muscle tissue, especially at the ECM. Besides, the postmortem evolution of muscle tissue significantly influenced adhesion to the ECM. This information is relevant to mitigate the contamination of meat, the food chain and ultimately human infection.

FEMS7-0663
Food Microbiology

GENOMIC EVIDENCE OF THE CLOSE RELATEDNESS OF FOOD, POULTRY, WILDLIFE AND HUMAN CLINICAL ISOLATES OF ESBL- PRODUCING ESCHERICHIA COLI O153:H10-A-ST10 EAE-BETA1

D. Diaz Jiménez[1], *Z. Zhou*[2], *A. Herrera Estévez*[1], *S. Viso González*[1], *M. Blanco Álvarez*[1], *L. Costoya Seco*[1], *A. Mora Gutiérrez*[1]
[1]*Universidad de Santiago de Compostela, Departamento de Microbioloxía e Parasitoloxía, Lugo, Spain*
[2]*University of Warwick, Warwick Medical School- Microbiology and Infection Unit, Coventry, United Kingdom*

Backgrounds

During the last decade, it has been widely recognized that the dissemination of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* is no longer restricted to the health care system but represents a growing problem involving food production and environmental integrity.

Objectives

In a ten-year surveillance study (2006-2016) on the presence of ESBL producing *E. coli* from human diarrhea, food, farm environment and wildlife in the north-west of Spain, we repeatedly detected an unusual atypical enteropathogenic *E. coli* O153:H10 ESBL-producing group. The aim of this study was to determine the degree of relatedness between human and other sources´ isolates.

Methods

Twenty representative isolates were characterized by conventional molecular typing, being all O153:H10-A-ST10 *fim*H54 *eae*-beta1 CTX-M-32/SHV-12 (19 and 1 isolates, respectively). By PFGE, the 20 clustered with ≥84.8%.

Additionally, 17 of the 20 isolates were whole genome sequenced. In the Minimum Spanning Tree of the core genome analysis based on the presence / absence of 2,513 genes included in the cgMLST scheme from Enterobase (https://enterobase.warwick.ac.uk/), the 17 genomes showed <40 differences (range 5-35) in relation to the human clinical isolate LREC-113. The number of SNPs in the core genomic regions present in 90% of the compared genomes was <62 for 13 of the 17. Interestingly, we detected 5 genomes in Enterobase showing 84-264 differences in the cgMLST comparison, carriers of *fim*H54 and *eae*-beta1 genes but non-ESBL producers.

Conclusions

Our results demonstrate that highly genomic related isolates of ESBL-producing EPEC O153:H10-A-ST10, implicated in human diarrhea, are presently spread in different niches of our region.

FEMS7-2300
Food Microbiology

ANTIMICROBIAL ACTIVITY FROM STREPTOCOCCUS LUTETIENSIS A45212 ISOLATED FROM POZOL, A FERMENTED MAIZE DOUGH

G. Diaz Ruiz[1], *S. Samano*[1], *C. Maldonado*[1], *C. Wacher*[1]
[1]*UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, ALIMENTOS Y BIOTECNOLOGIA, Mexico D. F., Mexico*

Backgrounds

In a previous study, a collection of antimicrobial-producing strains of *Streptococcu*s spp. isolated from Mexican pozol was identified. *Streptococcus lutetiensis* A45212, exhibited important antimicrobial activity against some pathogenic microorganisms by the well diffusion assay, being *L. monocytogenes* one of them.

Objectives

The purpose of the present study was to evaluate the antimicrobial effects of *S. lutetiensis* A45212 against *L. monocytogenes*, also to determine the suitability of low-cost carbon sources for antimicrobial production by *S. lutetiensis* A45212.

Methods

The antagonistic effect of the strain A45212 against *L. monocytogenes* was determined in MRS broth and also in a neutralized and concentrated cell free supernatant from the same culture medium. Molasees and whey were utilized as carbon sources in flask experiments.

Conclusions

In MRS broth, strain A45212 completely eliminated *L. monocytogenes* within 48h incubation at 30°C and in a neutralized and concentrated cell free supernatant of the strain, the bactericide effect against *L. monocytogenes* was only observed after 6h. Whey and molasses added in the culture medium proved to be suitable for both biomass and antimicrobial production by *S. lutetiensis* A45212 but the highest antimicrobial production was obtained in presence of whey medium.

FEMS7-2405
Food Microbiology

B-XYLOSIDASE ACTIVITY IN W. CONFUSA L9, PREDOMINANT IN POZOL FERMENTATION

G. Diaz Ruiz[1], M. López-Hernández[1], M.E. Rodríguez-Alegría[2], A. López-Munguía[2], C. Wacher[1]
[1]UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, ALIMENTOS Y BIOTECNOLOGIA, Mexico D. F., Mexico
[2]Instituto de Biotecnología- UNAM, Ingeniería celular y biocatálisis, Cuernavaca, Mexico

Backgrounds

Pozol is a Mexican refreshing, non-alcoholic acid beverage, consisting of a water suspension of hot-alkali treated and fermented maize dough. Although starch is the main carbohydrate available for the microbial development, it has been demonstrated that non-amylolytic lactic acid bacteria (NALAB) play an important role, such as particularly *Weissella* spp. is one of the predominant groups microorganisms during this fermentation.

Objectives

The aim of this work was to study *Weissella* spp. strains isolated from pozol from the carbon source perspective. In particular if *W. confusa* L9, a predominant NALAB, is able to grow on xylan or partially hydrolyzed xylan, also available in this fermentation environment.

Methods

Weissella confusa L9, *W. confusa* L17, *W. confusa* Snc40, *W. paramesenteroides* and *W. cibaria*, isolated from pozol, were used in this study. The carbohydrate consumption profile of each strain was obtained by Api 50CH; an enzymatic activity screening was also performed using Api Zym. Growth in xylan from beechwood was evaluated in all strains but only *W. confusa* L9 was chosen to grow with partially hydrolyzed xylan as carbon source. Xylooligosaccharides (XOS) analysis throughout fermentation time was performed by thin layer chromatography as well as high performance anion exchange chromatography with pulsed amperometric detection.

Conclusions

All *Weissella* strains evaluated showed interesting biochemical properties some related to pozol fermentation, such as xylose consumption, β-glucosidase and β-galactosidase activities, as well as different capacities to use xylan as the sole carbon source. Nevertheless, β-xylosidase activity was only found in *W. confusa* L9 since XOS consumption by this microorganism was demonstrated.

FEMS7-3139
Food Microbiology

CHARACTERIZATION OF BACILLUS CEREUS SPORES ISOLATED FROM AN ALGERIAN DAIRY PLANT AND THEIR ABILITY TO ADHERE TO STAINLESS STEEL SURFACE

N. Didouh[1], F. Pestellec[2], B. Moussa boudjemaa[3]
[1]Université Djilalli Bounaama. Khemis-Milliana. Algérie, biology, Tlemcen, Algeria
[2]ADRIA Developpement- UMT14.01 SPORE-RISK- Z.A. de Creac'h Gwen- F-29196- Quimper Cedex- France, ADRIA Developpement- UMT14.01 SPORE-RISK- Z.A. de Creac'h Gwen- F-29196- Quimper Cedex- France, Quimper, France
[3]Laboratoire de Microbiologie Appliqué à l'Agroalimentaire au Biomédical et à l'Environnement. Tlemc en-Algérie, Biology, Tlemcen, Algeria

Backgrounds

Bacillus cereus is an endospore-forming bacterium frequently found in dairy products and dairy environment.

Objectives

We investigated if the exosporium and spore surfaces, the length and the number of appendages were important for spore adhesion to the stainless steel surface.

Methods

In this study, molecular identification (M13 RAPD, Rep PCR,PFGE), the hydrophobicity and surface electrical charge of spores from fourteen (14) *Bacillus cereus* strains isolated from a dairy plant located in north-western Algeria (were studied using microbial adhesion to hydrocarbon (MATH) method, and zeta potential measurements, respectively.Four spore morphologies were investigated by transmission electron microscopy (TEM) after negative staining.The ability of spores to adhere to stainless steel was also studied.

Conclusions

The presence of an exosporium was not sufficient to explain the ability of spores to adhere to stainless steel surfaces. When physico-chemical surface characters of *B. cereus* spores were compared: the hydrophobicity, the appendages length, the surface of spore and exosporium were found as the significant adhesion parameters.

FEMS7-0293
Food Microbiology

GENOTYPING OF BACTERIAL ISOLATES FROM PIROTSKA "IRONED" SAUSAGE

S. Bogdanović[1], A. Jelušić[2], B. Danilović[3], T. Berić[2], I. Nikolić[2], S. Stanković[2], I. Dimkić[2]
[1]High Agrifood School of Professional Studies, Professional Veterinary, Prokuplje, Serbia
[2]Faculty of Biology - University of Belgrade, Microbiology, Belgrade, Serbia
[3]Faculty of Technology University of Niš, Food Technology and Biotechnology, Leskovac, Serbia

Backgrounds

The Pirotska "ironed" sausage is a traditional dry fermented product from south-eastern region of Serbia, made of different types of meat (e.g. horse, goat, sheep and beef) and spices, without additives or starter cultures. The flat sausage is a totally organic, unprocessed product without heat and smoke treatment applied.

Objectives

The aim of this study was to characterize 120 isolates of lactic acid bacteria (LAB) from Pirotska sausage produced by six different brands in two-year period using phenotypic and genotypic identification.

Methods

The phenotypic characterization and preliminary identification of LAB was based on general morphology and biochemical tests (gas production, growth at different temperatures, arginine hydrolysis etc.). The repetitive elements (REP, BOX, $(GTG)_5$-PCR) found in the genome of these bacteria and randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) using M13 primer were used for determination of genetic polymorphism. Sequencing of 16S rRNA gene was used for species level identification.

Conclusions

The 16S rRNA sequencing showed presence of only two species. *Lactobacillus sakei* was the dominant species (76%), followed by *Leuconostoc mesenteroides* (24%). However, plenty of genetic polymorphism within these two species was detected using $(GTG)_5$-PCR fingerprinting. The results after comparing fingerprinting patterns of bacterial populations from different brands, due to the use of different meat types as well as different percentages of meat content, revealed some genetic similarity in few clusters and emphasized significant polymorphism within others.

FEMS7-2883
Food Microbiology

INHIBITION OF ENTEROPATHOGENS BY LACTOBACILLUS AND BIFIDOBACTERIUM SPP. ISOLATED OF CHILDREN'S FECES

M. Santos do Carmo[1,2], E.P. Moraes Lopes[1], F.C. Morais Maia[1], C.C. Rocha[2], L.X. de Brito Rodrigues[1], C. R A V Monteiro[1], V. Monteiro-Neto[1,2]
[1]Ceuma University, Centro de Ciências da Saúde, São Luís, Brazil
[2]Federal University of Maranhão, Centro de Ciências Biológicas e da Saúde, São Luís, Brazil

Backgrounds

The diarrhea is responsible for 526,000 deaths annually in children under 5 years old. The classical approaches for its treatment such as electrolyte replacement and antibiotic therapy are not sufficient to solve all the deleterious effects generated to the organism. Thereby, the use of probiotic microorganisms is an innovative option for the regression or elimination of this infectious process.

Objectives

The goal of this study was to investigate the probiotic potential of microorganisms isolated from the feces of healthy children.

Methods

The methodology included phenotypic and molecular identification of bacteria, tolerance assays to the gastrointestinal tract (resistance to lysozyme, pH2 and 4, bile salts 0.5% and 1%), mucin adhesion, inhibition of pathogen adhesion to mucus and antagonism against enteropathogens EAEC 042, EHEC EDL933, EPEC E-2348/69, ETEC 1661-1 LT/ST+, Salmonella Choleraesuis INQS 028 and *Shigella flexneri* 2a.

Conclusions

Collectively, the species *B. bifidum* 14.2, *B.longum* subsp. *longum* 25.3, *B. longum* 49.3, *B.animalis* subsp. *lactis* 56.1 and *L. fermentum* 54.2 endured the gastrointestinal stress ($p<0.001$), adhered to the mucin similarly or more that the controls *L. fermentum* ATCC 23271 and *B. longum* subsp. *longum* ATCC 15707 ($p<0.001$ and $p<0.05$, respectively), inhibited the adhesion of the pathogens to gastric mucin in 77.17-99.83% ($p<0.001$) and produced antimicrobial substances. These data suggest that the isolated microorganisms are potential candidates for probiotics because they inhibited the main bacterial pathogens associated with moderate and severe childhood diarrhea and can represent an important strategy for the treatment of this infection.

FEMS7-2903
Food Microbiology

EVALUATION OF PROBIOTIC PROPERTIES OF BIFIDOBACTERIUM ISOLATES OF NEWBORNS FECES

V. Monteiro-Neto[1,2], E.P. da Silva Costa[1], M. Santos do Carmo[1,2], C. Regina Aragão Vieira Monteiro[1], E.P. Moraes Lopes[1], F. Carolina Morais Maia[1], L.X. de Brito Rodrigues[1], C. Cunha Rocha[1], M.R. Quaresma Bomfim[1]
[1]Ceuma University, Centro de Ciências da Saúde, São Luís, Brazil
[2]Federal University of Maranhão, Centro de Ciências Biológicas e da Saúde, São Luís, Brazil

Backgrounds

The normal microbiota of the newborns feces is composed of different genera of microrganisms, including *Bifidobacterium* species that play an important role in human health by regulating the pH of the large intestine by release lactic and acetic acid. Futhermore, there is evidence that they inhibit the growth of potential pathogens and putrefactive bacteria.

Objectives

The goal of the study was to investigate the probiotic potential of *Bifidobacterium* species isolated from feces of newborns, as well as to evaluate the period of intestinal colonization in three different moments of breastfeeding, including colostrum, transition milk and mature milk.

Methods

The methodology included mucin adhesion assay, tolerance assays to the gastrointestinal tract (resistance to lysozyme, pH2 and 4, bile salts 0.5% and 1%), *in vitro* antagonism of bacterial isolates against enteropathogens, molecular identification of bacterial isolates, and quantitative analysis by Polymerase Chain Reaction in (qPCR) of *Bifidobacterium* present in newborn feces

Conclusions

BF31, BF46 and BF51 isolates were found to be able to withstand conditions simulating the gastrointestinal tract, including acid pH and high concentrations of bile salts. In addition, these bacteria had a mucin adhesion capacity ($p < 0.05$) and inhibited enteropathogens of clinical relevance, with a mean variation of inhibition halos between 13.5 (± 2) mm and 25.5 (± 0, 7) mm. The isolates were identified as belonging to the *B. breve* by sequencing the 16S subunit rDNA. Quantification of bacterial DNA by qPCR showed a high estimate of *Bifidobacterium* spp. in newborn feces collected in all phases of breastfeeding (colostrum, transition and mature). These results suggest that these microrganisms play an important role in balancing the microbiota of newborns and that they are potential candidates for probiotics that can be used to control intestinal infections.

FEMS7-0799
Food Microbiology

SCREENING OF STRAINS ISOLATED FROM KOREAN TRADITIONAL FOOD AND EVALUATION ON BIOFUNCTIONAL EFFECTS OF GINSENG FERMENTED USING THESE STRAINS

S.J. Eom[1], H.S. Kim[1], J.E. Hwang[1], K.T. Kim[2], H.D. Paik[1]
[1]Konkuk university, Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea
[2]Konkuk university, Bio/Molecular Informatics Center, Seoul, Republic of Korea

Backgrounds

Panax ginseng Meyer is known as an Asian traditional herb that has diverse health benefits such as antiaging effects, immune regulation, prevention of cancer, and pain relief. A compound bioconverted by microorganisms (such as lactic acid bacteria) can have higher biofunctional activities and also has an advantage: it is nontoxic because it is isolated from food.

Objectives

The aims of this study were to screen lactic acid bacteria to find those suitable for ginseng fermentation and to evaluate the biofunctional effects of this fermented ginseng.

Methods

A total of 205 bacterial strains were isolated from Korean traditional foods including cabbage kimchi, doenjang, green onion kimchi, and gochujang and were used for screening. After the screening test for esculin hydrolysis and β-glucosidase activity, the selected strain was identified using 16S rRNA. A β-carotene assay, ex vivo NO assay, and MTT assay were conducted to evaluate beneficial effects of fermented ginseng extracts.

Conclusions

As a result, a strain isolated from cabbage kimchi showed the highest β-glucosidase activity (550 μM pNP/[min·mL]). Via analysis of 16S rRNA gene sequences, this isolate was identified as *Leuconostoc mesenteroides*. In β-carotene assays, fermented extracts showed 10% higher activity than did the nonfermented ones. In the case of MTT assays on HepG2 cells, cytotoxic activity of extracts fermented for 2 days (72%) was higher than that of nonfermented extracts (20%). Thus, ginseng extract fermented with lactic acid bacteria may serve as a nutraceutical agent for food industries.

FEMS7-0813
Food Microbiology

NITRIC OXIDE SCAVENGING OF GINSENG MARC EXTRACT FERMENTED BY PEDIOCOCCUS ACIDILACTICI IN YOGURT

S.J. Eom[1], J.E. Hwang[1], H.S. Kim[1], K.T. Kim[2], H.D. Paik[1]
[1]Konkuk university, Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea
[2]Konkuk university, Bio/Molecular Informatics Center, Seoul, Republic of Korea

Backgrounds

Panax ginseng is major commercial ginseng in Asia. Although the demand for ginseng has been continuously increasing, lots of by-products of ginseng (marc) have been scrapped or used as animal feed resources. Previously, we have reported that a *P. ginseng* by-product fermented with lactic acid bacteria has an antioxidant activity judging by ferric reducing power and a β-carotene bleaching assay. *Pediococcus acidilactici* is known to include representative strains of fermented dairy products and can inhibit growth of pathogens and cancer cells.

Objectives

The purpose of this study was to evaluate microbial enzymatic activities of *Pediococcus acidilactici*, changes in ginseng flavonoids, and nitric oxide scavenging activities of fermented milk supplemented with ginseng marc extract.

Methods

Ginseng marc was extracted with ethanol and lyophilized. Enzymatic activity of the microorganisms was determined by means of the API kit and flavonoid contents were measured by an aluminum chloride assay. Kaempferol served as a standard. Fermented milk was made by adding 5 g skimmed milk, 0.1% pectin, 1% ginseng marc, and 10^7 CFU/mL *P. acidilactici* to 500 mL of milk. Nitric oxide scavenging effects were measured by sodium nitroprusside.

Conclusions

As a result, a β-glucuronidase-induced carcinogenic substance was not detected in the *P. acidilactici*. After fermentation, flavonoid contents of the ginseng marc increased to 10.5 mg kaempferol per gram of solids. The viable cell count of fermented milk was 10^8 CFU/mL, and NO synthesis inhibition rate was 28.3% (control: 6.4%). According to these data, ginseng marc may be used in fermented food processing as a high-value functional material.

FEMS7-1319
Food Microbiology

THE ASSOCIATION OF SALMONELLA SHEDDING AND COLONIZATION WITH SINGLE NUCLEOTIDE VARIANTS IN THE PORCINE INNATE IMMUNE SYSTEM GENES

V. Farzan[1], M. Ainslie[2], R. Friendship[3], M. Jafarikia[4], C.F.M. de Lange[5], B. Lillie[2]
[1]University of Guelph, Population Medicine and Pathobiology, Guelph, Canada
[2]University of Guelph, Pathobiology, Guelph, Canada
[3]University of Guelph, Population Medicine, Guelph, Canada
[4]University of Guelph, Animal Biosceonces, Guelph, Canada
[5]University of Guelph, Animal Biosciences, Guelph, Canada

Backgrounds

Salmonella is commonly found on pig farms posing a food safety threat to humans due to direct contact with infected pigs, through consumption of contaminated pork, or via transmission to ground water and produce if the pig manure is used as land fertilizer. Genetic selection for resistance against *Salmonella* carrier-state can be considered as effective method to control *Salmonella* in pigs.

Objectives

The objectives of this study was to investigate the associations between single nucleotide variants (SNVs) in the porcine innate immune system genes and *Salmonella* shedding/colonization.

Methods

Fecal samples collected from 809 pigs 4 to 5 times from birth up to marketing, and tissue samples obtained at slaughter were cultured for *Salmonella*. DNA extracted from tissue samples were analyzed for SNVs in the several genes related to innate immune response. A multilevel mixed-effects logistic regression modelling method was used to analyze association between SNVs and *Salmonella* shedding and colonization.

Conclusions

Overall, 35 and 23% of pigs were tested positive on farm and at slaughter, respectively. SNVs analysis showed an association of on-farm *Salmonella* shedding with the mannan-binding lectin (MBL) 1-A (MBL1-A) variant (C/T), found in 18% of the study population (P= 0.02). In addition, the A variant allele of the nucleotide binding oligomerization domain containing 1 (NOD1-A), found in 63% of the study population, was associated with *Salmonella* colonization (P = 0.04). These findings indicate that *Salmonella* in pigs is controlled by genetic elements and can be used to breed the pigs that are resistance against *Salmonella* shedding and colonization.

FEMS7-2143
Food Microbiology

LACTOBACILLUS PLANTARUM B391 BACTERIOCIN EX-SITU STUDIES USING FRESH CHEESE AND PORK MEAT

D. Loureiro[1], J. Santos[1], V. Monteiro[1], C. Ramos[1], P. Fernandes[1]
[1]Instituto Politécnico de Viana do Castelo, Escola Superior de Tecnologia e Gestão, Viana do Castelo, Portugal

Backgrounds

Naturally occurring molecules able to inhibit the growth of food pathogens, as bacteriocins, are of great interest to the food industry due to their potential role in food safety.

Objectives

A bacteriocin produced by *Lactobacillus plantarum* B391, has been characterized and its use has been tested for the prevention of *Listeria monocytogenes* growth in cheese and pork meat to promote food safety in particular of products where *Listeria monocytogenes* is able to grow.

Methods

The effect on the reduction of an initial load of *Listeria monocytogenes* spiked on the food materials tested was determined by plating on ALOA agar. Bacteriocin B391 activity was determined using the spot-on-lawn method and successive dilutions.

Conclusions

The bacteriocin B391, a small peptide of 6KDa, showed to be very stable in a range of pH between 3.95 and 8.09. Its thermal stability is very high but pH dependent, being thermolabile at alkaline pH. In the present work, the activity of the purified bacteriocin was tested in fresh cheese and also in pork meat. In fresh cheese, when using an initial inoculum (10^5 CFU/ml) of *Listeria monocytogenes* in the presence of bacteriocin B391, more than 1 log of reduction of the initial population was obtained in a period of 2 months at 4ºC. The bacteriocin B391 was also used to obtain a coated plastic packaging film (PA/EVOH/PE). This film was tested in pork previously contaminated with *Listeria monocytogenes*, and it was possible to observe a reduction of 1 log after 24 h at 4ºC

FEMS7-0533
Food Microbiology

YEASTS FOUND IN BRINES USED IN THE SALTING STAGE OF MANCHEGO CHEESE

M. Fernández González[1], *R. Díaz Ceca*[1], *M. Arévalo Villena*[1]
[1]*University of Castilla La Mancha, Food Science, Ciudad Real, Spain*

Backgrounds

The salting stage during cheese processing is very important because regulates and selects the microbial populations and brine is a source that provides a great variety of yeasts to cheeses, although their population must be controlled in order to not cause problems in later stages.

Objectives

Identify the different yeast species that can be found in two types of "Manchego" type cheese brines made from both raw or pasteurized milk.

Methods

Serial dilutions of samples were seeded onto YPD (Yeast peptone dextrose) plates supplemented with diphenyl (150mg/L) and cloramphenicol (100mg/L) and incubated at 25ºC for three days. Yeast colonies were isolated from plates containing between 30 and 300 colonies and purified by streaking onto YPD. With the purpose of identifying the isolates to specie level, PCR (ITS-5.8 S rDNA)-RFLP was used and then one isolated of each molecular profile obtained was sequencied by using the domains D1/D2 and ITS region when necessary.

Conclusions

The microbial count was 2100 UFC/mL on the pasteurized cheese brines and 2900 UFC/mL on raw milk cheese. A total of 30 yeast colonies were isolated and 11 different species were identified. In both types of brines were isolated mainly *Debaryomyces hansenii (30%)* followed by *Candida pararugosa (20%), Pichia cactophila (10%) and Yarrowia lipolitica (6,7%).* Up to five different species were isolated exclusively in the brine of raw milk cheeses such as *Rhodotorula diobovata*, *R. mucilaginosa, Sporisorium cruentum, Filobasidium Uniguttulatum* and *C. zeylanoides*, and only two species were isolated in brines from pasteurized milk cheeses: *Kluyveromyces marxianus* and *C. atlantica*.

FEMS7-0542
Food Microbiology

ANTIMICROBIAL ACTIVITY OF SEVERAL YEAST SPECIES ISOLATED FROM FOOD AGAINST BACTERIAL PATHOGENS

M. Fernández González[1], *H.M. Gómez Gaona*[2], *A.I. Briones Pérez*[1]
[1]*University of Castilla La Mancha, Food Science, Ciudad Real, Spain*
[2]*University of Santander UDES, Industrial Microbiology, Bucaramanga, Colombia*

Backgrounds

In response to the increasing consumers demand for so-called minimal processing food with least amounts of chemical preservatives, it has begun to promote alternative methods of food preservation, such as the use of natural antimicrobials from microorganisms mainly lactic acid bacteria, but there are few studies of yeast antimicrobial activity against pathogenic bacteria in food.

Objectives

this work aims to study growth inhibition of five food pathogenic bacteria (*Salmonella enteritidis, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes* and *Clostridium perfringens*) by using 208 yeast strains from 18 different genera and more than 50 species, isolated from several food ecosystems and conserved at the University of Castilla La-Mancha yeast collection .

Methods

YPD (Yeast peptone dextrose) plates were inoculated by a sterile swab using a suspension of the pathogenic bacteria from 18 to 24 hours incubation adjusted to 1.5 x 10^6 bacteria/mL, and then 2.5 µL of yeast preculture standardized to 10^8 cells/mL was added to the plate. Plates were incubated at 30 ° C for 48 hours and finally, the inhibition halo was observed. For yeasts that showed antimicrobial activity against some pathogen, tests were performed at different pH (4.5, 5.5 and 6.5) and temperatures (20, 30, 37ºC).

Conclusions

90 yeast strains showed antimicrobial activity in varying degrees against any pathogens tested, being *S.aureus* and *C. perfringens*, more sensitive to yeasts action, Only *Metschnikowia pulcherrima, Kluyveromyces thermotolerans, Pichia galeiformes* and *P.caribbica* have inhibitory activity against the five pathogens. Further research on the potential of these strains as biological control agents is needed.

FEMS7-1258
Food Microbiology

HELICOBACTER PYLORI GROWTH/NO GROWTH PREDICTIVE MODEL IN REFERENCE MEDIA AND LETTUCE

M.C. Pina-Pérez[1], A. González[1], Y. Moreno[2], M.A. Ferrús[1]
[1]Universitat Politècnica de València, Biotecnología, Valencia, Spain
[2]Universitat Politècnica de València, Instituto de Ingeniería del Agua y Medio Ambiente IIAMA, Valencia, Spain

Backgrounds

Helicobacter pylori is one of the most widespread pathogens worldwide, causative agent of upper digestive tract diseases. It has been identified in raw and minimally processed foods. However, its isolation and quantification is really complex due to its capability to become into viable but non-culturable forms (VBNC), maintaining the infective potential. Predictive models are valuable mathematical tools to determine in which measure this microorganism could represent a risk, contributing to implement future reliable exposure assessment procedures.

Objectives

This work aims to fit by the first time the *H. pylori* growth/no growth kinetics in reference growth liquid broth and lettuce, under isothermal controlled conditions (37 ºC), by means the modified Gompertz equation.

Methods

Sterilized Brucella Broth supplemented with 5% (v/v) fetal bovine serum and filter sterilized lettuce extracts were inoculated with *H. pylori* 11638 NCTC to a final concentration of 1 x 10^3 CFU/mL, and incubated under microaerobic conditions. Culture absorbance was measured at 600 nm at regular intervals during 7 days. The viability of the culture was determined by plating in agar Dent at each sampling time.

Conclusions

Modified Gompertz equation accurately fitted the *H. pylori* growth in reference media (R^2 = 0.988; *RMSE* = 0.080). No significant growth was observed in lettuce, possibly due to variations of pH, nutrient availability, or additional components present in leafy extracts. In spite of this, the microorganism remained viable up to 5 days in lettuce.

This study has been supported by the Spanish Ministerio de Economía y Competitividad AGL2014/53875-R grant

FEMS7-1040
Food Microbiology

ALGERIAN EXTRA VIRGIN OLIVE OIL: ANTIBACTERIAL EFFECT

L. Firdousse[1], A. Tamendjari[2], N. Iaccarino[3], P. Rovellini[4], A. Randazzo[3]
[1]A. Mira University, Microbiology, Bejaia, Algeria
[2]A. Mira University, Biochemestry, Bejaia, Algeria
[3]University of Naples "Federico II", Pharmacy, Naples, Italy
[4]INNOVHUB-SSI- Via Giuseppe Colombo 79- 20133 Milano- Italy, INNOVHUB-SSI-, Milano, Italy

Backgrounds

Olive oil has a peculiar fatty acid composition and also contains several micronutrients and a variety of minor components responsible for its particular nutritional characteristics.

Objectives

Evaluation of antibacterial activity of different varieties of Algerian Extra virgin olive and their phenolic extracts

Methods

For antibacterial activity assay five extra virgin olive oils came from different Algerian varieties are used. The bacterial strains target three Gram negative (*Escherichia coli*, *Vibiro cholerea and Selmonella typhi*) and three Gram positive (*Bacillus subtilis*, *Staphylococcus aureus méthiciline résistant and Staphylococcus aureus*) with an initial inoculum 10^6 CFU/mL. The bactericidal activity assay was based on the method reported by Friedman et al 2003. To identify and quantify the phenolic compounds from various olive oils we using a reverse-phase high-performance liquid chromatography. Evaluation antibacterial activity for phenolic compound extracts is performed against same strains

Conclusions

Results revealed that the olive oils had a strong bactericidal action against a broad spectrum of microorganisms. Samples showed bactericidal activity, all Microorganisms target with an initial inoculum 10^6 CFU/mL did not survive after 1h of contact with olive oils. The analysis of individual phenolic compounds by HPLC reveals a similar qualitative composition which differs quantitatively depend the varieties oleuropein and ligstrosid derivatives were the phenolic compounds that statistically correlated with bacterial survival. These findings were confirmed by testing phenolic extracts of olive varieties against same bacterial.

These results indicate that not all oils classified as "olive oil" had similar bactericidal effects and that this bioactivity depended on their content of particular phenolic compounds.

FEMS7-2416
Food Microbiology

PHAGE BIOCONTROL OF SALMONELLA IN VITRO AND ON SPROUTING ALFALFA SEEDS

K. Fong[1], *S. Wang*[1]
[1]*University of British Columbia, Food- Nutrition & Health, Vancouver, Canada*

Backgrounds

Emerging *Salmonella* strains exhibiting antibiotic resistance represent a major challenge in food safety. Lytic bacteriophages were proposed as antimicrobials for mitigation of *Salmonella.*

Objectives

Specific objectives of our study were: (i) to investigate lysis profiles of bacteriophages isolated from British Columbia, Canada; (ii) to characterize four promising bacteriophage isolates on their morphological and genetic determinants; and (iii) to investigate their ability to inhibit *Salmonella* Enteritidis *in vitro.*

Methods

Salmonella phages (n=60) were isolated from irrigation water and sediment samples in BC. Host ranges were determined by spot-assay, using 20 outbreak serotypes, eight of which exhibited resistance to one or more antibiotics. Four broad-spectrum phages, SI1, SS1, SF1 and SS4, were further characterized by determination of genetic material (i.e., DNA or RNA), burst size and latent periods. Transmission electron microscopy (TEM) was performed for morphological classification. Finally, their efficacy to control *S.* Enteritidis, a serotype causing highest proportion of salmonellosis worldwide, was analyzed by conducting an *in vitro* spectrophotometric lysis assay.

Conclusions

The four phages lysed *S.* Enteritidis and seven of eight antibiotic-resistant strains. Burst sizes ranged from 20-83 phages/cell with short latent periods of 25-30 minutes. Nuclease digestion indicated a genome composed of DNA and TEM revealed phages belonging to *Siphoviridae* (i.e., non-contractile tails and ~200 nm length). Lastly, an *in vitro* lysis assay showed inhibition of *Salmonella* Enteritidis at a multiplicity of infection=100 over a 36-hour period.

These findings showed the promise of these phages in eliminating *Salmonella in vitro.* Future research should evaluate their lysing ability using a food model.

FEMS7-2806
Food Microbiology

PRODUCTION OPTIMIZATION AND CHARACTERIZATION OF ANTIFUNGAL COMPOUNDS FROM INDIGENOUS LACTOCOCCUS LACTIS SUBSP. CREMORIS PB6

M. Gajbhiye[1]
[1]*Tuljaram Chaturchand College, Microbiology, Baramati, India*

Backgrounds

Lactic acid bacteria are known for their use in biopreservation due to the ability of the production of several antimicrobial compounds.

Objectives

The major objective of this investigation was purification and characterization of antifungal compounds from an indigenous strain of *Lactococcus lactis* subsp. *cremoris* PB6.

Methods

Production optimization was done on the basis of pH, temperature and, static and stationary conditions.
Effect of pH, temperature and proteases on the antifungal activity of culture supernatant was determined. The antifungal compounds were concentrated by freeze drying and ultrafiltration followed by extraction with ethyl acetate. Antifungal compounds were isolated by silica gel column chromatography and further analysis was carried out by GC-MS.

Conclusions

Maximum antifungal activity was recorded at pH 5.5 and 6.5, and at temperature 30^{o}C on MRS agar and under stationary conditions. The activity of culture supernatant was pH dependent and resistant to the action of proteases. The activity was significantly reduced (P<0.05) with increase in temperature from 50 to 121^{o}C. Maximum antifungal activity was observed in the culture filtrates of 1 KDa membrane indicating their low molecular weight. Ethyl acetate extract gave the highest recovery of the antifungal compounds as the solvent extract from the ultrafiltrate showed strong antifungal activity. Two active fractions of antifungal compounds were obtained following silica gel column chromatography. GC-MS analysis revealed the presence of tetradecanoic acid and cyclo-(Leu-Pro). This is the first report of antifungal cyclo-(Leu-Pro) and tetradecanoic acid from *L. lactis* subsp. *cremoris*.

FEMS7-0990
Food Microbiology

EFFECTS OF EXPOSURE TO BIOCIDES ON SUSCEPTIBILITY TO ESSENTIAL OILS AND CHEMICAL PRESERVATIVES IN BACTERIA FROM ORGANIC FOODS

A. Galvez[1], R. Gadea[1], G. Nicolás[1], R. Pérez Pulido[1], O. Elena[1]
[1]University of Jaén, Ciencias de la Salud, Jaén, Spain

Backgrounds

In recent decades, reduced susceptibility of isolates to commonly-used biocides among important pathogenic bacteria genera has been observed, particularly in healthcare settings. This was often seen in association with an elevated frequency of antimicrobial resistances. The aim of this study was to determine the tolerance to essential oils and chemical preservatives in biocide-adapted bacterial isolates from organic foods.

Objectives

To determine the sensitivity of biocide-adapted bacterial strains to essential oils and chemical preservatives

Methods

A collection of 38 biocide-adapted strains with significant increases in their tolerance to biocides after step-wise exposure to these compounds were screened for sensitivity to essential oils and chemical preservatives.

Conclusions

Strains grown in presence of sublethal concentrations of quaternary ammonium compounds (benzalkonium chloride, hexadecylpyridinium chloride or cetrimide) showed a generalized increase in the sensitivity to preservatives. Similar results were found among hexachlorophene- or chlorhexidine-adapted strains. Moreover, tolerance to hexadecylpyridinium chloride showed a very strong positive correlation with 4-hydroxybenzoic acid, thyme oil and sodium nitrite increased susceptibility, as well as a strong correlation with clove oil, potassium sorbate and potassium nitrate increased sensitivities. On the contrary, an increase in the tolerance to preservatives among triclosan-adapted strains was detected.

Results from this study suggest that exposure of bacteria from foods to biocides is not always associated with co-selection for other antimicrobial resistances, especially against essential oils or chemical preservatives usually handled in the food industry, so these compounds could be used in food environments with no subsequent influence on the antimicrobial resistance selection along the food chain.

FEMS7-1060
Food Microbiology

EFFICACY OF PLASTIC FILMS ACTIVATED WITH NATURAL ANTIMICROBIALS AND HIGH PRESSURE TREATMENTS IN TWO MODEL FOOD SYSTEMS

A. Galvez[1], I. Ortega Blázquez[1], M.J. Grande Burgos[1], R. Pérez Pulido[1], A. Cobo[1], R. Lucas López[1]
[1]University of Jaén, Ciencias de la Salud, Jaén, Spain

Backgrounds

One of the alternatives to prevent the growth of pathogenic or altering microorganisms in foods is the use of films activated with antimicrobials, such as essential oils or bacteriocins, singly or in combination with high pressure hydrostatic treatments (HHP).

Objectives

To determine the efficacy of activated films in combination with HHP treatments on inactivation of foodborne pathogens.

Methods

Plastic films activated with different antimicrobials were tested against *Listeria innocua*. Then, fish fillets and fruit purees challenged with *L. innocua* were packaged in activated films and pressurized at 300 MPa. Viable counts of *Listeria* in the samples at 0, 3, 5, 7 and 10 days of incubation at 4 °C were determined.

Conclusions

Films with greater antimicrobial activity against *L. innocua* were those activated with carvacrol and thymol plus the enterococcal bacteriocin enterocin AS-48. A remarkable decrease in the counts was detected after 24h for the combination of thymol and bacteriocin. In fish samples, the most effective treatment was the combination of the activated film (thymol plus bacteriocin) with HHP treatments. In the fruit samples, no *Listeria* were detected from day 5, in none of samples treated. The results suggest that the combination of antimicrobials either alone or with HHP treatments would be of great interest for increasing bactericidal activity, reducing the amount of antimicrobials and minimizing the impact of thymol on the food organoleptic properties.
Acknowledgements: This work was supported by grant UJA2014/06/25 (U of Jaén).

FEMS7-1004
Food Microbiology

HIGH PREVALENCE OF MCR-1 IN ESCHERICHIA COLI ISOLATES FROM DIARRHOEIC PIGLETS IN SPAIN, ASSOCIATION WITH ST10 AND ST131 CLONES

V. García Menéndez[1], I. García Meniño[1], S.C. Flament-Simon[1], M.P. Alonso García[2], J.E. Blanco Álvarez[1], J. Blanco Álvarez[1]
[1]Universidad de Santiago de Compostela, Microbioloxia e Parasitoloxia, Lugo, Spain
[2]Hospital Universitario Lucus Augusti, Microbiology, Lugo, Spain

Backgrounds

Antimicrobial resistance is one of the most important threats in food-production animals since it can lead to spread of resistant bacteria to humans. The emergence of colistin resistance, an antibiotic used in pig production to prevent infections those mainly produced by *E. coli*, and particularly the current finding of plasmid encoded *mcr*-1 colistin resistance gene, has aggravated this worldwide problem.

Objectives

This study focuses on the detection of *mcr-1* gene in *E. coli* isolates from diarrhoeic piglets recovered in Spain during the years 2006-2016, and on the molecular typing of positive *mcr-1* isolates.

Methods

A total of 536 isolates were examined by PCR for the presence of *mcr-1*, obtaining 140 (26,1%) positives isolates of which 82 were further characterized.

All isolates, except one, showed colistin MIC of ≥4 mg/ml and by serotyping it was detected high diversity of O:H serotypes (35), being O157:HNM, O25b:H4 and O141:H4 the most commons. ETEC (37), STEC (4), ETEC/STEC (5) and aEPEC (23) pathotypes were identified by detection of LT, STa, STb, Stx1, Stx2, Stx2e toxins and the *bfp*A gene.

According to phylogenetic grouping, isolates were distributed in phylogroups A (50), B1 (16), B2 (7) and E (9). Different clonotypes (25) and ST (20) were detected by sequencing *fumC-fimH* alleles and MLST respectively, identifying CH11-24 and ST10 as the commonest followed by CH4-24, ST29 and CH40-22, ST131.

Conclusions

Our results highlight the high prevalence of colistin resistance in diarrogenic isolates from swine in Spain and the important association with ST10 as well as with the successful ST131 clones.

FEMS7-2194
Food Microbiology

MYCOTOXIN-PRODUCING FUNGI IN DIFFERENT STAGES OF MAIZE PRODUCTION CYCLE

M. García-Díaz[1], B. Patiño[1], C. Vázquez[1], M.T. González-Jaen[2], J. Gil-Serna[1]
[1]Universidad Complutense de Madrid, Facultad de Biologia- Microbiología III, Madrid, Spain
[2]Universidad Complutense de Madrid, Facultad de Biologia- Genética, Madrid, Spain

Backgrounds

Maize (*Zea mays* L.) is one of the main cereals as a source of food, feed and processed products for industry. Contamination by mycotoxins represents a significant risk to human and animal health.

Objectives

The objective was to evaluate the presence of mycotoxin producing fungi in order to detect the moment when contamination occur during maize production cycle.

Methods

Maize samples were evaluated in two different plots located in Madrid (Spain). Three different stages were sampled: Anthesis (R0, 25 male flowers and 25 female flowers), kernel dent (R5, 25 cob) and physiological maturity (R6, 3 kg of grain). DNA extraction was performed after 24 h of incubation at 28 ºC in Sabouraud-cloramphenicol broth and species-specific PCR protocols were carried out to detect the most important mycotoxigenic *Aspergillus* and *Fusarium* species. The results obtained were confirmed using morphological identification.

Conclusions

The aflatoxin-producing *Aspergillus flavus* was detected in all staged sampled indicating that contamination occur in the earlier period of the production cycle. In R5 stage, many new species were detected including fumonisin (*Fusarium proliferatum* and *F. verticillioides*) and trichothecene producers (*F. sporotrichioides*) as well as ochratoxin A producing species (*A. tubingensis*). All these species were also detected in the last step evaluated together with *A. welwitschiae*, potential ochratoxin A producer. The knowledge of the moment when contamination occurs is crucial to stablish a correct treatment schedule to prevent mycotoxin entering the food chain.

FEMS7-0472
Food Microbiology

SYNERGISTIC EFFECT OF A HEAT TREATMENT IN PRESENCE OF INDIVIDUAL COMPOUNDS OF ESSENTIAL OILS IN THE INACTIVATION OF HYPER-RESISTANT STRAINS OF ESCHERICHIA COLI

E. Geens[1], D. Berdejo[2], R. Pagán[2], E. Gayán[1], A. Aertsen[1], C. Michiels[1], D. Garcia-Gonzalo[2]
[1]KU Leuven- Laboratory of Food Microbiology,
Department of Microbial and Molecular Systems M2S- Faculty of Bioscience Engineering, Leuven, Belgium
[2]Instituto Agroalimentario de Aragón IA2, Universidad de Zaragoza-CITA, Zaragoza, Spain

Backgrounds

Heat treatments in presence of individual compounds (ICs) of essential oils have shown a synergistic lethal effect in food preservation treatments. However, it is unknown whether the presence of stress-resistant variants in bacterial populations might jeopardize the efficacy of food preservation combined treatments.

Objectives

The objective of this investigation was the evaluation of the lethal effect of a combined process (heat + ICs) against genetic stress-resistant variants of *Escherichia coli* MG1655.

Methods

The strains used were wild-type (WT) and its variants selected for IC (CAR, CIT, LIM), heat (DVL1) or HHP resistance (DVL10 and LMM1020). These strains also developed cross-resistance to heat.

Suspensions were independently treated at 53ºC in presence or absence of 200 ppm of ICs: carvacrol, citral or cinnamaldehyde (MES buffer pH 5.3; $3 \cdot 10^7$ CFU/mL). Survivors were plated in TSA and incubated at 37ºC for 24h.

Conclusions

Individual application for 10 min of heat or ICs treatments caused less than 1 $\log_{10}$ cycle reduction of populations of any strain. Simultaneous application of heat in presence of each IC showed a synergistic lethal effect against WT, causing the inactivation of more than 5 $\log_{10}$ cycles of bacterial population. The synergistic effect of these combined processes against stress-resistant variants was variable (1-4 $\log_{10}$ cycles), but remarkably a heat treatment in presence of carvacrol inactivated more than 5 $\log_{10}$ cycles of the initial population of every strain. This study shows the synergistic lethal effect of a combination of heat and carvacrol against not only WT strain, but also against genetic stress-resistant strains.

FEMS7-2659
Food Microbiology

INCIDENCE OF LISTERIA MONOCYTOGENES IN RETAILED CHEESES AND CHARACTERIZATION OF PERSISTENT STRAINS IN TWO CZECH PRODUCERS

T. Gelbíčová[1], Z. Tomáštíková[1], A. Pietzka[2], W. Ruppitsch[3], R. Karpíšková[1]
[1]Veterinary Research Institute, Department of Bacteriology, Brno, Czech Republic
[2]Austrian Agency for Health and Food Safety, National Reference Laboratory for Listeria, Graz, Austria
[3]Austrian Agency for Health and Food Safety, Institute of Medical Microbiology and Hygiene, Vienna, Austria

Backgrounds

The ability of *L. monocytogenes* to persist in different environments is the main factor contributing to food contamination. Ripened cheeses are risk commodity regarding *L. monocytogenes* contamination and their consumption has been implicated with human listeriosis outbreaks.

Objectives

The study was focused on the finding of incidence and characteristics of *L. monocytogenes* in retailed cheeses with respect to their safety for human.

Methods

The detection and enumeration of *L. monocytogenes* in 387 samples of ripened and steamed cheeses was carried out at the time of purchase and at the end of the shelf-life. The obtained isolates were characterized by serotyping, multilocus sequence typing (MLST), macrorestriction analysis and sequencing of the *inlA* and *inlB*. The selected persistent strains were analysed using core genome (cg) MLST.

Conclusions

L. monocytogenes was detected in 5.2% originally packaged cheeses from different producers and none sample exceeded the limit of 100 CFU/g. The possibility of *L. monocytogenes* isolates to cross protective host barriers was verified by sequencing of *inlA* and *inlB*. Persistent *L. monocytogenes* strains were found in blue-veined cheeses (serotype 1/2a, pulsotype 719) and smear-ripened cheeses (1/2a/713) from two producers. Persistent strains had the same sequence type ST204 and showed very similar pulsotypes in contrast with non-persistent strains. The clonal similarity of persistent strains was confirmed also on the basis of cgMLST. The persistent strains may carry some specific characteristics which enable their adaptation for surviving in the cheese-processing.

Acknowledgements: This study was carried out with financial support of AZV 16-31488A, QJ1210300 and QK1710156.

FEMS7-1149
Food Microbiology

EFFECT OF NATIVE FOOD MICROFLORA ON THE DETECTION OF PATHOGENIC YERSINIA ENTEROCOLITICA AND YERSINIA PSEUDOTUBERCULOSIS

E.T. Gensberger[1], L. Antonielli[1], A. Sessitsch[1], T. Kostic[1]
[1]AIT Austrian Institute of Technology GmbH, Center for Health & Bioresources, Tulln, Austria

Backgrounds

Yersiniosis, mainly caused by *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, was ranked as the third most commonly reported foodborne disease in Europe since 2008-2015 [EFSA 2016, 14(12):4634]. However, the reported numbers are almost certainly an underestimation, due to the fact that the recovery using the standardized method (ISO 10273:2003) has been reported as suboptimal. Accordingly an action has been called for at the EU level [EFSA 2007, 595] to improve current methods for detection of pathogenic *Yersinia* spp. in food.

Objectives

Our study was based on the hypothesis that native food microflora plays a key role in the enrichment and thus detection efficiency of pathogenic *Yersinia* spp. from food, and that better understanding of the microbial ecology of the enrichment is essential for its adaptation and improvement.

Methods

Diverse food samples including meat and meat products, dairy products and fresh produce that were inoculated with high and low concentration of *Y. enterocolitica* (biovar 1A, 2, 4) and *Y. pseudotuberculosis* were investigated. Enrichment was performed using both selective (ISO 10273:2003) and non-selective broths and detection was assessed using selective agars. In total 3078 samples were analyzed, and the determined detection efficiency for *Yersinia* spp. was between 0% and 100%. Interestingly, the performance of enrichment method differed for the food sample analyzed, indirectly confirming our hypothesis about the influence of native microflora. In order to better understand the microbial community composition and its changes in relation to enrichment procedure, a comprehensive sequencing analysis using Illumina 16S rRNA (V3-V4 region) amplicon sequencing was performed. Microbial communities were analyzed in 846 selected samples that showed high and low recovery of *Yersinia* species. As a reference non-spiked enriched and native food samples were included.

Conclusions

The observed differences in the microbial community composition should allow identification of inhibitory microflora providing valuable basis for the development of more efficient detection methods.

FEMS7-3202
Food Microbiology

ESTABLISHING THE ANTIBACTERIAL EFFECT AND KINETICS OF ACTION OF RANDOM PEPTIDE MIXTURES AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

V. Ghate[1], T. Stern[1], R. De Leo[2], Z. Hayouka[1]
[1]The Hebrew University of Jerusalem - The Faculty of Agriculture, Institute of Biochemistry- Food Science and Nutrition, Rehovot, Israel
[2]University of Modena and Reggio Emilia, Life Sciences, Modena, Italy

Backgrounds

Antibiotic resistance is a growing problem in food and agriculture. To combat antibiotic resistance, interventions need to be designed cleverly to outsmart microbes. One such intervention is the use of random peptide mixtures. Random peptide mixtures are mixtures of two or more peptides that may be random in their sequence but are highly controlled in their stereochemistry.

Objectives

1. To determine the antibacterial effect of five different random peptide mixtures against methicillin-resistant *Staphylococcus aureus* (MRSA).
2. To establish the kinetics of action of these mixtures.

Methods

Five 20-mer mixtures – L-leucine-L-lysine (LK), L-phenylalanine-L-lysine (FK), L-phenylalanine-D-lysine (F^DK), L-ornithine-L-leucine (OL) and L-tryptophan-L-lysine (WK) were synthesized using Fmoc-assisted solid phase peptide synthesis. A culture of MRSA was incubated with these mixtures at 37 °C for 24 h, at concentrations ranging from 1.563 to 200 µg/mL. The fate of the bacterial cells was monitored by tracking the absorbance of the culture at 595 nm. Subsequently, the mixtures were ranked on the basis of their minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values. To elucidate the kinetics of the antibacterial effect, the Gompertz model was fitted to the growth curves.

Conclusions

OL was the most potent random peptide mixture, with bactericidal effects observed 50 µg/mL onwards and a bacteriostatic action taking place at lower concentrations. The next most effective mixture was LK, followed by FK, F^DK and WK. Going forward, this study will elucidate the mechanism of action of these mixtures by shedding light on their effect on critical cellular targets.

FEMS7-2304
Food Microbiology

ASPERGILLUS NIGER AGGREGATE SPECIES ON SPANISH FOOD MATRICES AND THEIR PUTATIVE ABILITY TO PRODUCE MYCOTOXINS

J. Gil Serna[1], M. García-Díaz[1], C. Vazquez[1], M.T. Gonzalez-Jaen[2], B. Patiño[1]
[1]Complutense University, Microbiology III, Madrid, Spain
[2]Complutense University, Genetica, Madrid, Spain

Backgrounds

Aspergillus niger aggregate contains at least 11 species which are morphologically indistinguishable but vary widely in their ability to produce mycotoxins.

Objectives

The objective was to determine the most important *A. niger* aggregate species in Spain as well as their potential mycotoxin-producing ability.

Methods

More than 180 strains included in *A. niger* aggregate were isolated from several sources including cereals, grapes and pulses among others. Identification of the most common species (*A. tubingensis*, *A. niger* and *A. welwitschiae*) were performed using species-specific PCR protocols. When no positive amplification was obtained, a partial region of the calmodulin gene was sequenced. Moreover, the putative ability of the isolates to produce ochratoxin A (OTA) and fumonisin B2 (FB2) was checked testing the presence of the genes involved in their biosynthesis by PCR.

Conclusions

72% of the isolates obtained were identified as *A. tubingensis*, followed by *A. niger* (16%) and *A. welwitschiae* (8%). Only 3 isolates were sequenced and identified as *A. brasiliensis*. Presence of the complete specific OTA cluster was only found in two isolates (one *A. niger* and one *A. welwitschiae*) demonstrating that OTA production is not widespread in *A. niger* aggregate. All *A. niger* and 10% of *A. welwitschiae* isolates presented the complete cluster of genes involved in FB2 biosynthesis. The rest of *A. welwitschiae* isolates only contained *fum1*, *fum19* and *fum15*. Therefore, the presence of *A. niger* aggregate species in Spanish food product does not seem to suppose a real risk of OTA contamination and only *A. niger* appears to be related to FB2 contamination.

FEMS7-3200
Food Microbiology

OCCURRENCE OF FUSARIUM TOXINS IN OATS MARKETED IN SPAIN

J.V. Gómez[1], A. Tarazona[1], J.V. Gimeno-Adelantado[2], M.A. García-Esparza[3], R. Mateo-Castro[2], M. Jiménez[1], E.M. Mateo[1]
[1]Universitat de Valencia, Microbiología y Ecología, Burjassot, Spain
[2]Universitat de Valencia, Química Analítica, Burjassot, Spain
[3]Universidad CEU-Cardenal Herrera, Farmacia, Moncada, Spain

Backgrounds

The health-beneficial properties of oats (dietetic properties, high beta-glucan content and anticarcinogenic effects) have led to an increase in consumption of oats and oat-based food products in the recent years. Contamination of this cereal with toxigenic *Fusarium* spp. is a global problem resulting in important economic losses. In addition, some *Fusarium* spp. produce mycotoxins, such as fumonisins (FUMs), zearalenone (ZEA) and trichothecenes, such as deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxins. These fungal metabolites are usually related to esophageal cancer, oestrogenic activity or gastrointestinal problems, DNA and RNA fragmentation and protein synthesis inhibition in both humans and animals.

Objectives

The aim of this study was to determine the presence of FUMs (FB1 + FB2), ZEA, DON, NIV, and T-2/HT-2 toxins in oats marketed in Spain.

Methods

Mycotoxins were determined by optimized and validated analytical chromatographic methods.

Conclusions

These mycotoxins were detected in 11%, 10%, 14%, 7% and 19% of the oat samples, respectively, at levels below European Union limits in all cases. The obtained results were compared with the presence of possible toxigenic *Fusarium* spp. responsible for their biosynthesis. However, the co-occurrence of different toxins in some samples suggested that synergistic activity of these mycotoxins should be evaluated. The main *Fusarium* spp. detected were members of the *Sporotrichiella*, *Liseola*, and *Discolor* sections. Correlation was found between the presence of certain *Fusarium* species and mycotoxin contamination in the same sample. This is the first report on the occurrence of *Fusarium* toxins in oats in Spain.

FEMS7-3104
Food Microbiology - Part II

EFFECT OF (SUB)LETHAL LEVELS OF FOOD-RELATED STRESSES (BIOCIDES AND PROCESSING TREATMENTS) ON THE EMERGENCE OF ANTIMICROBIAL RESISTANCE THROUGHOUT THE FOOD CHAIN

A. Alvarez-Molina[1], D. Fernández[1], C. Andrés[1], M. López[1], M. Prieto[1], A. Alvarez-Ordóñez[1]
[1]Universidad de León, Food Hygiene and Technology, León, Spain

Backgrounds

The latest report on Antimicrobial Resistance (AMR) by EFSA/ECDC shows a trend towards an increase in the detection of foodborne pathogens with multi-drug resistance (MDR) and the appearance of AMR to some antibiotics which are the last effective treatment for MDR infections.

Objectives

This study assessed the contribution of disinfection and food processing treatments to the generation of AMR. We aimed at isolating AMR mutants of *Salmonella spp, Listeria monocytogenes* and *Escherichia coli* after serial (sub)lethal treatments of Ultraviolet (UV) light, non-thermal atmospheric plasma (NTAP) and biocides (peracetic acid, benzalkonium chloride, sodium hypochlorite).

Methods

Five strains of *Salmonella* spp., three of *L. monocytogenes* and four of *E. coli* were grown overnight in BHI and exposed to repeated cycles (10 cycles) of treatment at various (sub)lethal intensities. After each cycle, survivors were recovered and grown in fresh broth to initiate a new treatment cycle, and an aliquot was stored under freezing. Frozen samples were grown overnight in BHI and spread plated on Mueller-Hinton agar plates supplemented with inhibitory concentrations (established for the wild-type strains) of ampicillin, cefotaxime, ciprofloxacin, erythromycin, gentamycin, streptomycin, tetracycline, colistin and vancomycin. Resistant variants were pheno-typically characterized in terms of their resistance against various clinically relevant antibiotics.

Conclusions

Our results confirm that disinfection practices and food processing technologies used in food industries may select for AMR microorganisms that pose a serious risk to the food chain. Whole Genome Sequencing of the most relevant strains will be carried out in the future to identify mutations responsible for the AMR phenotypes.

FEMS7-2717
Food Microbiology - Part II

MONITORING TETRACYCLINE RESISTANCE IN CHEESE BY FUNCTIONAL METAGENOMICS

A.B. Florez Garcia[1], *L. Vazquez*[1], *B. Mayo*[1]
[1]*IPLA-CSIC, Departamento de Microbiología y Bioquímica, Villaviciosa-Asturias, Spain*

Backgrounds

Metagenomics techniques have been successfully applied for monitoring antibiotic resistance genes in environmental, animal, and human ecosystems. However, in spite of the food chain being claimed to be a key player in the spread of antibiotic resistances, metagenomics approaches have been scarcely used in foods.

Objectives

In this work, we report on the prevalence and evolution of tetracycline resistance (Tc^r) determinants in a blue-veined, raw milk cheese by functional metagenomics.

Methods

The same cheese batch was sampled at manufacturing (day 3) and ripening (day 60) stages. Dilutions were plated in the presence of tetracycline on non-selective and selective conditions for lactic bacteria. DNA from cultures in the counting plates was then isolated and used to construct four fosmid libraries. Clones were analysed by restriction enzyme digestion, PCR and sequencing.

Conclusions

Four different Tc^r genes were identified among 300 clones, *tet*(A), *tet*(L) *tet*(M), and *tet*(S). *tet*(A), *tet*(M), and *tet*(S) were detected in libraries at day 3 and *tet*(L) was the single gene identified in the two libraries at day 60, suggesting an evolution of the Tc^r gene types along with that of the microbial populations during ripening. Six representative clones were completely sequenced. All Tc^r genes characterized in this study resided on plasmids and/or were flanked by insertion sequences, mobilization- and conjugation-associated protein-encoding genes.

- Metagenomic techniques can be a valuable tool to evaluate the resistome of cheeses and other fermented dairy products.
- Raw-milk made cheeses should be considered as reservoirs of Tc^r genes with a high potential for horizontal transference.

FEMS7-3250
Food Microbiology - Part II

ECOLOGICAL ANTIFUNGAL AGENTS AND OCHRATOXIN A PRODUCTION BY A. STEYNII AND A. TUBINGENSIS IN OAT GRAINS

A. Tarazona[1], J.V. Gómez[1], J.V. Gimeno-Adelantado[2], M.A. García-Esparza[3], R. Mateo-Castro[2], M. Jiménez[1], E.M. Mateo[1]
[1]Universitat de Valencia, Microbiología y Ecología, Burjassot, Spain
[2]Universitat de Valencia, Química Analítica, Burjassot, Spain
[3]Universidad CEU-Cardenal Herrera, Farmacia, Moncada, Spain

Backgrounds

Oats constitutes a nutritious and popular cereal with high beta-glucan content. Beta-glucans are prebiotics and new oat-based functional food products have been developed. However, cereals are the first source of ochratoxin A (OTA) in the diet and OTA is a potent nephrotoxic, teratogenic, immunosuppresive and carcinogenic compound. The most important ochratoxigenic *Aspergillus* spp in cereals are *A. steynii*, followed by *A. ochraceus* and *A. westerdijkiae* from section *Circumdati* and *A. tubingensis* followed by *A. niger* complex (especially *A. niger* and *A. welwitschiae*, which is considered a phylogenetic cryptic species contained in *A. niger*) from section *Nigri*. Application of chemical fungicides is the most widely used strategy to control fungal growth and OTA biosynthesis in cereal crops. The health risks associated with fungicide residues in foods are considered less relevant than the potential impacts of mycotoxins on public health.

Objectives

The aim of this study was to assess the activity of two ecological antifungal agents (copper oxychloride and sulfur) and a non-ecological fungicide (mancozeb) on the control of OTA biosynthesis by *A. steynii* and *A. tubingensis* in oat grains under different environmental conditions linked to climate, such as relative humidity and temperature.

Methods

OTA was determined by an optimized/validated method involving liquid chromatography with fluorescence detection.

Conclusions

Temperature, fungicide dose, and time significantly influenced ($P<0.05$) OTA accumulation in oats. Sub-inhibitory doses of antifungal agents can enhance OTA production. Mancozeb proved more effective that the other chemicals to prevent OTA production under similar conditions.

FEMS7-0884
Food Microbiology - Part II

PREVALENCE OF LISTERIA MONOCYTOGENES IN POULTRY DURING PROCESSING IN SLAUGHTERHOUSE AND IN POULTRY MEAT PRODUCTS

E. Gonzalez-Fandos[1], *I. Perez-Arnedo*[1]
[1]*University of La Rioja, Food Technology, Logroño, Spain*

Backgrounds

Meat and poultry products are often identified as the source of foodborne pathogens. Raw poultry is a well-recognized source of *L. monocytogenes*, and many surveys have confirmed the presence of this pathogen on fresh poultry. Some authors have associated cases of listeriosis with the consumption of undercooked chicken

Objectives

The pesent study was conducted to determine the prevalence of *Listeria monocytogenes* during slaughter and processing of poultry.

Methods

Broiler carcasses were examined at selected stages of slaughter: after defethering, after evisceration, after washing and after chilling. Also carcasses and fresh poultry legs, breast and wings were collected in the processing plant after cutting.

Conclusions

Listeria monocytogenes was not detected in any sample after defeathering. After eviscerati uon *L. monocyogenes*was detected in 13.3% of the carcasses examined. A higher percentage of carcasess were *L. monocytogenes* positive after washing (43.33%). After chilling, this pathogen was detected in 33.3% of carcasses. In processing plant, *L. monocytogenes* was detected in 22% of carcasses, 84% of legs, 88% of breasts and 100%, of wings .*L. monocytogenes* counts were below 2 log cfu/g in all the samples analyzed.

Higher prevalence of *L. monocytogenes* was observed in poultry meat products (legs, breasts and wings) than in carcasses. Since carcasses were *L. monocytogenes* negative at defeathering stage and percentage of carcasses contaminated with *Listeria* increase in the late stages of processing, it can be conclude that carcass were contaminated during slaughter and handling.

FEMS7-0900
Food Microbiology - Part II

EFFECTIVENESS OF IMMERSION TREATMENTS WITH LACTIC AND ACETIC ACIDS AND MODIFIED ATMOSPHERE PACKAGING AGAINST CAMPYLOBACTER JEJUNI IN POULTRY

E. Gonzalez-Fandos[1], *I. Perez-Arnedo*[1]
[1]*University of La Rioja, Food Technology, Logroño, Spain*

Backgrounds

Raw poultry is a well-recognized source of *Campylobacter jejuni.*

Objectives

The aim of this study was to evaluate the combined effect of a mixture of lactic and acetic acids and packaging in modified atmospheres on the growth of *Campylobacter jejuni* on poultry legs stored at 4ºC.

Methods

Fresh chicken legs were inoculated with *Campylobacter jejuni*. After the inoculation, the chicken legs were dipped into a mixture containing 1% lactic acid (v/v) and 1% acetic acid (v/v). Control legs were treated with distilled water. Inoculated samples were packaged under different gas mixtures: vacuum, 20%CO_2 / 80%N_2, 40%CO_2/ 60% N_2 or air.

Conclusions

Significant differences ($p<0.05$) in mesophiles and psychotrophs counts were found between the legs treated with a mixture of lactic and acetic acid and the control legs after treatment. The lowest mesophiles counts were observed in those samples treated and packaged in 40%CO_2/ 60% N_2. Legs washed with a mixture of 1% lactic acid and 1% acetic acid solution showed a significant ($p<0.05$) inhibitory effect on *Campylobacter jejuni* compared to control legs, being about 1.35 log units lower in the first ones than in control legs after treatment. No significant reduction on *Campylobacter jejuni* was observed in samples packaged under vacuum, 20%CO_2 / 80%N_2 or 40%CO_2/ 60% N_2 .

In conclusion, immersion of chicken legs in a mixture of 1% lactic acid and 1% acetic acid solution can reduce *Campylobacter jejuni* populations on fresh poultry. Atmospheres containing 20%CO_2 / 80%N_2 or 40%CO_2/ 60% N_2 are not able to reduce *Campylobacter jejuni*.

FEMS7-0584
Food Microbiology - Part II

SULPHITE TOLERANCE REQUIRES PROPER AUTOPHAGIC FUNCTION IN SACCHAROMYCES CEREVISIAE

E. Valero[1], J. Tronchoni[2], R. Gonzalez[2], P. Morales[2]
[1]Universidad Pablo de Olavide, Dpto. Biología Molecular e Ingeniería Bioquímica, Sevilla, Spain
[2]ICVV CSIC-Universidad de La Rioja-Gobierno de La Rioja, Enología, Logroño, Spain

Backgrounds

Sulphur dioxide (often dosed as bisulphite salts) has had widespread use in winemaking for centuries. It is used at several stages of the process, and for several purposes, mainly as antioxidants and as antimicrobials, even before the onset of fermentation. Sulphites are also used as preservatives in many canned and processed foods. Its usefulness during wine fermentation depends on the relatively high tolerance to sulphites of industrial wine strains of the yeast *Saccharomyces cerevisiae*. Sulphite tolerance of these yeast strains is related to mutations that promote high level transcription of *SSU1*, the gene coding for a plasma membrane sulphite pump required for efficient sulphite efflux.

Objectives

This work was designed, in order to better understand sulphite cellular targets in yeast, as well as microbial sulphite resistance.

Methods

By competition of bar-coded Yeast Knockout (YKO) collections we have identified some of the cell functions that are more specifically required in order to survive sulphite insult.

Conclusions

Our results show increased sulphite sensitivity for most KO strains involving genes required for autophagy, so pointing to this process as a key one in the detoxification of sulphite, probably through recycling of damaged proteins. Conversely, some deletion yeast strains, that were impaired under no-stressed conditions, recovered some competitiveness in the presence of sulphite. These functions are probably specifically sensitive to sulphite, so that already defective strains do not suffer from an additional impairment in the presence of sulphite.

Acknowledgments: MINECO(AGL2015-63629-R, RTC-2014-2186-2) EU(7FP-IRSES-GA612441); Junta de Andalucía(P10-AGR6544)

FEMS7-2192
Food Microbiology - Part II

SALMONELLA FUNCTIONAL GENOTYPING: PREDICTING PHENOTYPIC TRAITS FROM WHOLE GENOME SEQUENCES

J. Goris[1], P. Scheldeman[1], H. Pouseele[1], K. De Bruyne[1]
[1]Applied Maths, N.V., Sint-Martens-Latem, Belgium

Backgrounds

Surveillance of food-borne illnesses such as salmonellosis has shifted from microbial genotyping to Whole Genome Sequencing (WGS) over the last couple of years. Not only does WGS enable high-resolution strain typing via e.g. whole genome MLST or SNP analysis, it also holds the potential to replace or complement conventional methods for antimicrobial susceptibility testing, virulence profiling and serotyping.

Objectives

We developed and validated a *Salmonella* genotyping plugin for the BioNumerics® software, resulting in a push-button application detecting antibiotics resistance genes, virulence genes and predicting *Salmonella* serotypes starting from WGS data.

Methods

The *Salmonella* genotyping plugin uses public databases for serotype, virulence and resistance prediction. Virulence and resistance prediction tools start from the assembled genomic sequences and use a BLAST-based approach to detect and identify the genes of interest. Serotypes are determined directly on the raw reads, using an implementation of SeqSero (Zhang et al., 2015 J. Clin. Microbiol. 53(5), 1685-1692) on the BioNumerics® calculation engine.

A large set of publically available WGS data from food-related *Salmonella enterica* isolates were analyzed with the *Salmonella* genotyping plugin and results were validated with traditional typing information.

Conclusions

The *Salmonella* functional genotyping plugin proofs to be an accurate predictive tool for *Salmonella* phenotypes, implemented/integrated on the BioNumerics® platform next to the read quality assessment, (de novo) assembly algorithms, and wgMLST and wgSNP tools This user friendly WGS workflow provides accurate pathogen identification, antimicrobial resistance profiling and virulence factor determination, possibly improving transmission tracking and biological risk assessment in food-borne *Salmonella* outbreaks.

FEMS7-3245
Food Microbiology - Part II

LACTOBACILLUS REUTERI STRAINS FOR NATURALLY PREVENT CAMPYLOBACTER COLONIZATION IN CHICKEN

A. Greppi[1], P. Tetteh Asare[1], C. Schwab[1], C. Lacroix[1]
[1]Institute of Food- Nutrition and Health, Department of Health Sciences and Technology HEST, Zurich, Switzerland

Backgrounds

Campylobacter is a commensal of chickens infecting entire flocks at early age, and Campylobacteriosis is the most frequently reported food-borne illness in the EU. Reuterin is a antimicrobial system produced by *Lactobacillus reuteri* glycerol dehydratase activity from glycerol. In aqueous solution at physiological conditions, reuterin consists of 3-HPA, its hydrate and dimer, and acrolein, which was identified as the active antimicrobial component. *L. reuteri* forms natural biofilms in the crop.

Objectives

Here, we want to exploit reuterin formation of chicken isolates of *L. reuteri* to reduce *Campylobacter* contamination *in vivo*.

Methods

In a first step, *Lactobacillus* strains were isolated from crop and feces of chicken using modified MRS, species identity was confirmed using species specific 16S rRNA gene PCR and reuterin formation potential was detected using PCR targeting *pduC*. A total of 200 strains were isolated from 4 different farms in Switzerland, 26 of them were confirmed as *L.reuteri*, 14 were pduCDE positive. RepPCR was applied to identify clonal isolates. Reuterin production during incubation of *L. reuteri* and during growth in 500 mM glycerol solution and mMRS supplied with 100 mM was quantified by IC-PAD and HPLC. Inhibition activity of reuterin were tested on a large panel of *Campylobacter* spp. isolated from chicken and human infections. *L. reuteri* isolates were also tested for antibiotic resistance against tetracycline, erythromicyin and chloramphenicol.

Conclusions

In conclusion, a collection of characterized *L. reuteri* strains isolated from chicken was established. They represent a ground for application of *L. reuteri* produced reuterin to prevent/reduce colonization of enteropathogens in poultry.

FEMS7-2358
Food Microbiology - Part II

BACTERIOPHAGE-MEDIATED ELIMINATION OF SALMONELLA FROM RAW MILK

B. Grygorcewicz[1], X. Stachurska[1], J. Laskowska[1], P. Nawrotek[1]
[1]West Pomeranian University of Technology- Szczecin- Faculty of Biotechnology and Animal Husbandry, Department of Immunology- Microbiology and Physiological Chemistry, Szczecin, Poland

Backgrounds

Salmonella enterica , especially serovars such as Enteritidis and Typhimurium, are major causes of global food-borne diseases. The transmission of *Salmonella* could also be linked with raw or filtered milk and products made from them. Bacteriophages could be applied as an effective, alternative agent for biocontrol of these pathogens.

Objectives

The aim of study was the evaluation of the lytic activity of bacteriophage sall_v2 against *Salmonella* Enteritidis and *Salmonella* Typhimurium and application of the phage as a biocontrol agent in the *in vitro* studies.

Methods

Bacteriophage was isolated from wastewater using *Salmonella enterica* ssp. *enterica* sv. Enteritidis ATCC 13076 as a host. The morphology, host range, pH and thermal stability as well as the adsorption rate and one step growth of the isolated phage was evaluated by plaque assay using the double agar layer method. *The lytic activity of bacteriophage in Lysogeny broth medium was assessed* by measurements of optical density (OD) at wavelength 600 nm. In-milk activity of bacteriophage was evaluated by culture methods.

Conclusions

This study indicates that isolated bacteriophage can effectively reduce the quantity of *Salmonella* in raw milk. Bacteriophages used as the biocontrol agent could reduce the risk of food-borne salmonelloses associated with milk and products made from the raw, filtered, and un-pasteurised milk, such as cheeses.

FEMS7-3074
Food Microbiology - Part II

IDENTIFICATION OF ENTEROBACTERIACEAE BACTERIA IN FRESH FRUITS AND VEGETABLES AND THEIR RESISTANCE TO ANTIBIOTICS AND CHLORHEXIDINE.

N. Guizani[1], Z. Al-Kharousi[1], I. Al-Bulushi[1], A. Al-Saadi[2]
[1]Sultan Qaboos University, Food Science and Nutrition, Muscat, Oman
[2]Sultan Qaboos University, Crop Sciences, Muscat, Oman

Backgrounds

Many outbreaks due to consumption of fruits and vegetables are caused by bacteria belonging to Enterobacteriaceae which are considered among the most potent and prevalent pathogens that have acquired resistance to most antibiotics.

Objectives

Objectives

To identify isolated bacteria from fresh produce, screen them for their resistance to antibiotics and to chlorhexidine and identify some antibiotic or disinfectant resistance genes in these bacteria.

Methods

Enterobacteriaceae count was performed using violet red bile glucose agar. Bacteria were identified by Polymerase Chain Reaction targeting bacterial 16S rRNA gene. Bacteria were screened for their resistance to antibiotics using disc diffusion method. Susceptibility of isolates to chlorhexidine was investigated by determining the minimum inhibitory concentration (MIC) using broth microdilution method.

Conclusions

Fresh produce marketed in Oman contain diverse bacteria including opportunistic pathogens and antibiotic-resistant bacteria. Enterobacteriaceae bacteria showed resistance to chloramphenicol (1%), resistance/intermediate resistance to ampicillin (63%), cephalothin (54%), amoxicillin/clavulanic acid (32%), cefoxitin (29%), tetracycline (10%), kanamycin (7%), nalidixic acid (7%) and trimethoprim (7%) and intermediate resistance to ertapenem (1%) and imipenem (3%). Four types of ampC β-lactamase genes (CIT, EBC, FOX and MOX) and three Tetracycline resistance genes of tet(A) and tet(K) were detected in 9 bacteria. All bacteria were susceptible to chlorhexidine. The MIC of chlohexidine ranged from 1 to 64 µg/ml which is below the recommended concentration in food production areas (100-200 µg/ml). The resistance genes of qac EΔ1, qacE and qacG which can confer resistance to various biocides including chlorhexidine were detected in some bacteria as well as the integron integrase IntI 1 gene.

FEMS7-1367
Food Microbiology - Part II

MOLECULAR CHARACTERSATION OF VIM-1 PRODUCING E. COLI IN GERMAN LIVESTOCK

A. Irrgang[1], J. Hammerl[1], B.A. Tenhagen[1], M. Grobbel[1], S. Schmoger[1], K. Daehre[2], N. Roschanski[2], U. Rösler[2], A. Käsbohrer[1]
[1]German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany
[2]Freie Universität Berlin, Institut für Tier und Umwelthygiene, Berlin, Germany

Backgrounds

In 2015, a VIM-1 carbapenemase-producing *E. coli* was detected in the colon content of a pig at slaughter in Germany. Later, additional *E. coli* isolates were obtained from another slaughter batch of the same farm. Molecular characterization revealed close phylogenetic relationship of the isolates. Interestingly, in one isolate the *bla*$_{VIM-1}$ integron seemed to be integrated in the genome, whereas other isolates carried *bla*$_{VIM-1}$ also on a plasmid.

Objectives

In this study, molecular characterisation of selected isolates will be presented.

Methods

Faecal samples, boot swabs and environmental samples of the swine fattening farm were used for isolation of bacteria according to the EFSA protocol for ESBL detection. VIM-1-producing *E. coli* were characterised by PFGE, Southern Blot hybridisation, MLST and NGS.

Conclusions

bla$_{VIM-1}$ *E. coli* were isolated from samples of different barns. In two isolates, *bla*$_{VIM-1}$ is located on a plasmid whereas other isolates harbour *bla*$_{VIM-1}$ on the chromosome. Results of the WGS proofed the localisation of the class 1 integron and provided some hints explaining the genome stability for more than four years.
VIM-1-producing *E. coli* are present in German pig farming. Persistence in one farm over a period of at least five months and transmission between different barns of the farm was observed. Further investigations were conducted to identify the route of entry and ways of transmission.

FEMS7-1083
Food Microbiology - Part II

NOVEL STRESS SURVIVAL ISLET SSI-2 IN LISTERIA MONOCYTOGENES ST121 SUPPORTS SURVIVAL UNDER ALKALINE AND OXIDATIVE STRESS

E. Harter[1], E.M. Wagner[1], A. Zaiser[1], S. Halecker[1], M. Wagner[1], R. Kathrin[1]
[1]University of Veterinary Medicine, Institute for Milk Hygiene- Milk Technology and Food Science, Vienna, Austria

Backgrounds

Listeria (L.) monocytogenes is able to survive environmental stresses and to persist in food processing environments. Two genes from non-pathogenic *L. innocua*, *lin0464* and *lin0465*, have been found to be inserted in a hypervariable genetic hotspot in *L. monocytogenes*. Lin0464 is a putative transcriptional regulator and Lin0465 an intracellular pfpI protease. Strains devoid of *lin0464-lin0465* harbor either the stress survival islet 1 (SSI-1) or the gene *LMOf2365_0481*.

Objectives

PCR screening revealed that strains of sequence type (ST) 121 predominantly harbor *lin0464-lin0465*. To elucidate the function of Lin0464 and Lin0465, non-polar deletion mutants were exposed to distinct stress conditions.

Methods

While oxidative and alkaline stress resulted in decreased survival of the *lin0465* deletion mutant, acidic, cold and salt stress did not have any effect. In parallel, deletion of *lin0464* decreased survival under oxidative and alkaline stress. Additionally, we could show that constitutive expression of *lin0465* but not of *lin0464* in strain F2365, harboring the *LMOf2365_0481* insert, resulted in increased survival under oxidative and alkaline stress. qRT-PCR revealed increased expression of both genes under oxidative stress conditions after 10 minutes at 20°C in a σ^B-independent manner. Furthermore, we detected that the expression of *lin0465* is regulated by the transcription factor *lin0464*.

Conclusions

This indicates that *lin0464* and *lin0465* are a functional unit. In conclusion we could identify a new stress survival islet 2 (SSI-2), predominantly present in *L. monocytogenes* ST121, which is involved in alkaline and oxidative stress response and could support survival and persistence of *L. monocytogenes* in the food processing environment.

FEMS7-1014
Food Microbiology - Part II

ADAPTIVE RESPONSE OF LISTERIA MONOCYTOGENES BIOFILMS TO A DEHUMIDIFICATION STRESS

J. Esbelin[1], C. Chambon[2], D. Viala[2], M. Hebraud[1,2]
[1]French National Institute for Agricultural Research, Centre Auvergne-Rhône-Alpes- UMR MEDiS- Site de Theix, Saint-Genès Champanelle, France
[2]French National Institute for Agricultural Research, Centre Auvergne-Rhône-Alpes- PFEMcp- Site de Theix, Saint-Genès Champanelle, France

Backgrounds

Listeria monocytogenes is a foodborne pathogen able to adhere and form biofilms on various surfaces. Associated with a high mortality rate, it is one of the major biological concerns in food hygiene. Since few years, industries attempt to reduce the environmental impact of hygiene operations in the workshops of refrigerated food processing, through optimized use of dehumidification after cleaning disinfection treatments

Objectives

Our study was focused on the adaptive response of *L. monocytogenes* cells, growing in biofilms, to a dehumidification stress mimicking food plants conditions.

Methods

The intracellular and surfaceome subproteomes were analyzed by shotgun proteomics through three complementary extraction methodologies (enzymatic shaving, biotinylation and cell fractionation). *L. monocytogenes* EGD-e and L028 biofilms were grown on stainless steel discs at 25°C during 24h, pre-adapted to 10°C and placed in a desiccation chamber where the air Relative Humidity was stabilized to 75%. The different subproteomes were analyzed after 3h and 24h dehumidification by comparison with non-stressed biofilms.

Conclusions

Among the surface proteins identified, 21 and 29 were differentially expressed during dehumidification for EGD-e and L028, respectively. Three of them were common to both strain, an autolysin, Iap and an ABC transporter. Among intracellular proteins, 38 and 65 were differentially expressed in EGD-e and L028 respectively. Five proteins were common to the two strains. The majority of these proteins belongs to information pathway (35%) and intermediary metabolism (25%) functional categories. These results could contribute to a better comprehension of mechanisms involved in the resistance and persistence of this pathogen in food plants despite the daily hygiene procedures.

FEMS7-0345
Food Microbiology - Part II

SELECTION OF METABOLIC BIOSENSORS FOR SCREENING OF LACTOCOCCUS LACTIS CELLS WITH INCREASED PRODUCT-YIELD FOR INDUSTRIAL FERMENTATIONS

J. Hernandez-Valdes[1], *A. Solopova*[1], *O.P. Kuipers*[1]
[1]*GBB, Molecular Genetics, Groningen, The Netherlands*

Backgrounds

Some molecules are desired products for the food industry and are by-products of fermentation by many microorganisms. One example of these compounds is diacetyl, a volatile aromatic compound, giving a buttery flavor, associated with dairy products, and produced by species of the lactic acid bacteria (LAB) family. The identification of individual cells that produce an increased amount of diacetyl is complicated with existing technologies.

Objectives

Even though there are previous studies with engineering strategies to increase its production, the yields are low. Thus, in this project we aim to develop a high throughput method for identification of existing cells with increased product-yield, based on a recently established protocol to culture individual microbial cells in micro-droplets of emulsions.

Methods

The first step is to select the indicator strains that bear fluorescent reporter constructs under regulation of suitable promoters that are activated by diacetyl. To accomplish this, we tested the toxicity to diacetyl in *Lactococcus lactis* MG1363, and performed a transcriptional analysis with the maximum tolerable concentration of diacetyl added during exponential growth. In this way we identified candidate promoters responding to diacetyl.

Conclusions

We observed up-regulation of the genes in the riboflavin operon (*ribDBAH*). Subsequently, we amplified the promoter region ($P_{ribDBAH}$) and cloned it upstream of the *gfp* gene in the pSEUDO integration vector. Preliminary results with the *L. lactis* strain (MG1363 pseudo10 gene::$P_{ribDBAH}$-*sfgfp(Bs*)) in microtiter plate assays and flow cytometry show an increased GFP expression, proportional to serial concentrations of diacetyl. This potential biosensor strain for diacetyl is now under further characterization.

FEMS7-1307
Food Microbiology - Part II

CROSS SECTIONAL STUDY OF HEPATITIS E VIRUS IN SPANISH PIG SLAUGHTERHOUSES

M. Hernández[1], D. Rodriguez-Lazaro[2], A. Navarro[3], L. Domínguez[3], J. Goyache[3], N. García[3]
[1]Instituto Tecnológico Agrario de Castilla y León ITACyL,
Laboratory of Molecular Biology and Microbiology, Valladolid, Spain
[2]University of Burgos,
Departamento de Biotecnología y Ciencia de los Alimentos. Área de Microbiología, Burgos, Spain
[3]VISAVET Health Surveillance Centre - UCM, Neglected and Emerging Diseases Unit, Madrid, Spain

Backgrounds

Hepatitis E virus (HEV) represents the main cause of enterically transmitted hepatitis worldwide, mostly by drinking contaminated water and by the ingestion of infected pork meat. HEV is a single-stranded positive-sense RNA virus which is increasingly being recognized as the cause of zoonotic acute liver disease in many western countries.

Objectives

We report serological and PCR data of the analysis of HEV in individual samples (faeces, liver and serum) taken between July to October 2015 in 20 different Spanish pig slaughterhouses located in 29 provinces that represents the 50% of national pig production, to estimate the prevalence of the virus in Spain.

Methods

RNA extraction from 244 faeces and 241 livers was performed using QIAamp® viral RNA mini kit and RNeasy Midi kit (QIAGEN) and analysed by RT-PCR (Jothikumar *et al.*, 2006). 241 serum samples were tested using the ID Screen Hepatitis E multi species indirect ELISA (ID.vet).

Conclusions

From a total of 726 samples analysed, 19,67%, 21,99% and 71,37%, resulted positive in liver, faeces and sera samples, respectively. Results indicate that the virus is circulating among most of the Spanish swine hut, and a significant percent of animals (almost 20%) were indeed infected when the sample was taken, as the virus RNA was present in liver as well as in faeces. HEV should no longer be considered of little importance in Spain and further studies on the circulation of the virus in both domestic and wild animals (swine, wild boar, deer), including the whole food chain, are needed.
Acknowledgement: EFS-INIA-RTA2014-00024-C04.

FEMS7-1254
Food Microbiology - Part II

CAN FRESH PRODUCE BE PROTECTED FROM COLONISATION BY HUMAN PATHOGENS THROUGH THE APPLICATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AS BIOCONTROL?

A. Holmes[1], M. Kalamara[2], N. Stanley-Wall[2], N. Holden[1]
[1]The James Hutton Institute, Cell and Molecular Science, Dundee, United Kingdom
[2]University of Dundee, College of Life Sciences, Dundee, United Kingdom

Backgrounds

The consumption of fresh produce, such as lettuce and tomato, is increasingly being linked to foodborne outbreaks of verotoxigenic *Escherichia coli* and *Salmonella enterica*. Strategies need to be implemented to improve food safety by reducing the risk of infection from ready to eat (RTE) horticultural products. One strategy would be to restrict the colonization of fresh produce by human pathogens through the pretreatment of crops with PGPR.

Objectives

A number of commercially available PGPR treatments were tested in an *in vitro* competition assay with *E. coli* O157:H7 isolate Sakai. PGPR which were able to out-compete Sakai *in vitro* were tested under glasshouse conditions.

Methods

PGPR were applied to lettuce seedlings at transplant and a second application after seven days. Fourteen days after transplant, lettuce plants were challenged with 10^7 cfu/ml *E. coli* O157:H7 Sakai by root-soak. Colonisation of Sakai on lettuce roots was measured after seven days by viable count on selective media.

Conclusions

Bacillus amyloliquefaciens strain GB03 (commercially available as Companion®) and a naturally occurring *Pseudomonas putida* isolate SCRI_5304 were able to outcompete *E. coli* O157:H7 Sakai *in vitro.* However, when tested in glasshouse conditions, there is no significant difference in the numbers of *E. coli* O157:H7 strain Sakai recovered from PGPR treated versus untreated lettuce roots.

Application of PGPR, which are effective in competition against *E. coli* O157:H7 Sakai *in vitro,* are not able to reduce colonisation of human pathogens *in planta*. Continued research into controls and treatments are required to reduce the risk of foodborne illnesses arising from RTE produce.

FEMS7-1857
Food Microbiology - Part II

PARTIAL 16S RRNA SEQUENCING IS USEFUL METHOD FOR SPECIES IDENTIFICATION OF STAPHYLOCOCCI ISOLATED.

L. Hoveida[1], S. Rostami[2], M. Halaji[3]
[1]Department of Microbiology, Falavarjan Branch - Islamic Azad University, Isfahan, Iran
[2]Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Department of Microbiology - School of medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Backgrounds

Some species of *staphylococci* isolated are considered technologically important in the manufacturing processes of various meat-derived products, especially dry fermented sausages which can potentially cause food poisoning.

Objectives

The aim of this study was, using partial 16S rRNA sequencing for the species identification of *staphylococci* isolated and determining antimicrobial susceptibility pattern of *Staphylococcus* isolated from sausages.

Methods

During august 2015 to march 2016, 61 sausages samples were collected and were sub-cultured on Giolitti- Cantoni broth and Baird-Parker agar media. Isolates were identified based on morphological and physiological characteristics of *Staphylococci* spp. Partial 16sRNA sequencing was used for species identification of *Staphylococci* isolates. Furthermore, antimicrobial susceptibility test of *Staphylococci* isolates was performed by the Disc diffusion method on Mueller– Hinton agar according to CLSI guideline.

Conclusions

We detected Staphylococci spp. in 26 different samples. These identified isolates were belonged to 9 different species and included: 5 (19.5) S. vitulinus, 5 (19.5) S. epidermidis, 3 (11.5) S. pasteuri, 3(11.6) S. saprophyticus 2 (7.7) S. warneri, 2(7.7) S. succinus, 3 (11.5) S. aureus, 2 (7.7) S. equorum, 1(3.8) S. gallinarum,. About 88.5% of species were assessed negative for coagulase test. The most sensitivity isolates were toward levofloxacin and ciprofloxacin and gentamycin each with 100%. Also, the highest antibiotic resistances among isolates were observed against penicillin (73.1%) and tetracycline (42.3%).

In conclusion, 16srRNA sequencing was an objective and accurate method for proper identification of *Staphylococci* species. Further studies on molecular characterization of resistant *staphylococci* isolated from meat-derived products should be carried out.

FEMS7-1556
Food Microbiology - Part II

IT IS POSSIBLE TO INCREASE THE FOLATE CONTENT OF A TRADITIONAL CEREAL-BASED FERMENTED FOOD USING LACTIC ACID BACTERIA SELECTED FOR THEIR FOLATE SYNTHESIS CAPACITY

F. Bationo[1,2], Y. Hemery[1], L. Songre-Ouattara[2], F. Hama[2], F. Saubade[1], I. Berrazega[1], Z. Almousa Almaksour[1], J.P. Guyot[1], C. Humblot[1]
[1]Institut de Recherche pour le Developpement, Nutripass, Montpellier, France
[2]Institut de Recherche en Sciences Appliquées et Technologies, Département Technologie Alimentaire, Ouagadougou, Burkina Faso

Backgrounds

Folate deficiency is still common in developing countries as well as in many industrialized contexts. Beneficial effects of mandatory food fortification programs have already been demonstrated but concerns still exist over their possible adverse effect. To improve folate content of fermented foods, it is also possible to use *in situ* fortification through fermentation. This has been validated in dairy products, but data on cereal-based fermented foods are still scarce even if these types of foods are highly consumed around the world.

Objectives

The objective of this work is to produce a traditional cereal-based fermented food made from pearl millet enriched into folate through fermentation. This product is consumed by all population in different countries of West Africa, especially by at risk population such as young children.

Methods

We have studied the ability to produce folate into a collection of 150 lactic acid bacteria isolated from traditional fermented food made from pearl millet and called ben-saalga. After detection of genes coding enzymes involved in folate biosynthesis pathway, we checked the production of folate in culture medium. We used the most active strains to inoculate ben-saalga in order to increase its folate content.

Conclusions

We were able to increase the folate content of the pearl millet porridge using the most active strains. This work is an example of the use of the diversity of microorganisms naturally present in cereal-based fermented food in improve the nutritional quality of the traditional food. This strategy is now used in different countries, representing various nutritional contexts.

FEMS7-0200
Food Microbiology - Part II

BROAD IN VITRO INHIBITION OF FOOD-BORNE PATHOGENS, ANTIMICROBIAL-RESISTANT, AND SPOILAGE BACTERIA BY MARINILACTIBACILLUS PSYCHOTOLERANS ISOLATED FROM CHEESE SMEAR

N. Bianco[1], N. Javorka[1], E. Arias-Roth[1], J. Hummerjohann[1]
[1]Agroscope Institut für Lebensmittelwissenschaften ILM, Bacteriological Food Safety, Bern, Switzerland

Backgrounds

Protective cultures are an innovative tool to control pathogens, antimicrobial resistant (AMR) and spoilage bacteria in food.
We have previously shown anti-listerial activity of *Marinilactibacillus psychotolerans*, a Gram-positive rod isolated from natural cheese smear, in cheese-ripening experiments.

Objectives

Here, we aimed to measure the *in vitro* activity of *Marinilactibacillus psychotolerans* strains against a broad range of food-borne pathogens, spoilage and AMR bacteria in order to develop an effective protective culture in the future.

Methods

20 different Gram-positive and Gram-negative strains including methicillin-resistant *S. aureus* (MRSA), *Proteus*, ESBL-positve *E. coli*, *L. monocytogenes* were used as indicators. The inhibition potential of 2 genetically-different protective cultures of *Marinilactibacillus psychotolerans* FAM208060 (already used in the cheese exoeriments) and FAM23170 (new isolate) against these bacteria were tested in quadruplicate. The *in vitro* inhibition test was conducted at 12 °C during 96 hours after inserting 10^9 cfu/ml protective culture in wells punched in BHI soft agar inoculated with 10^6 cfu/ml indicator strain and the extent of the inhibition zone was measured.

Conclusions

The results showed inhibition of all strains tested by both *Marinilactibacillus psychotolerans* strains. Strain FAM23170 always exhibited stronger inhibition than FAM20860. Comparative analysis showed that different strains of MRSA, *Proteus*, and *Morganella* were statistically significantly stronger inhibited than *Listeria innocua* FAM20870, of which inhibition has already been described in cheese-ripening experiments.
In conclusion, the obtained *in vitro* data are showing the potential of *Marinilactibacillus psychotolerans* to be used as an effective protective culture with broad-spectrum activities against pathogens, spoilage and AMR bacteria, in particular MRSA.

FEMS7-1101
Food Microbiology - Part II

SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) PREVALENCE AND VIRULENCE GENES IN CATTLE AND SHEEP

B. Oporto[1], M. Ocejo[1], A. Hurtado[1]
[1]NEIKER-Tecnalia, Department of Animal Health, Derio, Spain

Backgrounds

Ruminants are sources of food-borne infections by Shiga toxin-producing *Escherichia coli* (STEC) in humans.

Objectives

To investigate the prevalence of STEC in cattle and sheep in the Basque Country (northern Spain), and assess the potential health risk of livestock-carried STEC isolates according to their virulence gene profile.

Methods

Rectal faeces collected between February 2014 and June 2016 from 301 farms (115 dairy sheep, 104 beef, 82 dairy cattle) were pre-enriched in modified TSB-novobiocine. *E*. *coli* O157 was isolated after immunomagnetic separation and chromogenic isolation, and non-O157 STEC were recovered from lactose-positive colonies in MacConkey agar. Isolates were identified and characterized by amplification of *stx*1, *stx*2, *eae*A and E-*hly*A genes. Serotype O157:H7 was identified by PCR amplification of *rfb*E and *fli*C genes and serogroups O5, O26, O91, O103, O111, O121 and O145 with primers targeting the *wzx* or *wzy* genes.

Conclusions

STEC were isolated from 63.5% of beef cattle herds, 56.5% of sheep flocks and 30.5% of dairy cattle herds. Prevalence of STEC O157:H7-positive herds was 18.3% in beef cattle, 20.0% in sheep and 14.6% in dairy cattle. Serogroups O26, O103, O111 and O145 were never detected, and O5, O91 and O121 were isolated in a small proportion of herds/flocks. Most O157:H7 isolates (96.5%) had the toxigenic profile *stx*2+*eae*A+E-*hly*A, which was very uncommon in non-O157 STEC isolates (4.4%). Proportion of herds/flocks that harboured non-O157 STEC isolates carrying the *eae*A gene and thus considered potentially pathogenic to humans, was 3.8% in beef cattle, 7.3% in dairy cattle and 7.0% in sheep.

FEMS7-2866
Food Microbiology - Part II

LACTOBACILLUS CASEI N87 ADAPTED UNDER ANAEROBIC VS RESPIRATORY CONDITIONS AS A MICROBIAL STARTER IN FERMENTED SAUSAGES PRODUCTION.

L. Iacumin[1]
[1]*University of Udine, Department of Agriculture- Food- Environmental and Animal Science, Udine, Italy*

Backgrounds

Lactic acid bacteria had a central role in fermented sausages (FS). *Lactobacillus casei* is used in several fermented foods, such as dairy, bakery and fermented meat products. This microorganism can be used as starter to ferment meat and to improve health benefit, considering the possibility to use this matrix as a probiotic carrier. The adjunction of *L. casei* strains, adapted under respiratory (R) vs anaerobic conditions (A), can affect the physiological response of cells, influencing growth, survival and capabilities of meat fermentation.

Objectives

Investigation of the effects of the anaerobic and respiratory conditions of growth, prior to inoculation, on *L. casei* N87 during fermented sausages ripening.

Methods

Three trials were conducted and compared. A) FS with no starter addition; b) FS inoculated with *Lactobacillus casei* N87 adapted under A; FS inoculated with *Lactobacillus casei* N87 adapted under R. The evolution of microflora, chemical/physical parameters (pH, a_w, weight loss, colour), production of volatile compounds, antioxidant capabilities, proteolysis and survival of the strains, were evaluated.

Conclusions

Results demonstrated similarities in the major part of the chemical/physical parameters. Different concentrations of the inoculated strains were observed during ripening, and this probably affected the proteolysis, which was the most significantly different parameter monitored at the end of the ripening period.

Acknowledgements: This work was funded by Ministero dell'Istruzione, dell'Università e della Ricerca, Rome, Italy, FIRB n. RBFR107VML.

FEMS7-1475
Food Microbiology - Part II

IN VIVO ASSESSING OF NANOMETRIC CHANGES ON THE SURFACE OF WHOLE TOMATOES INOCULATED WITH CANDIDA GUILLIERMONDII YEAST USING ATOMIC FORCE MICROSCOPY

E.D.P. Infante Luna[1]
[1]*Universidad Distrital Francisco José de Caldas, Facultad de Ciencias y Educación, Bogotá, Colombia*

Backgrounds

Studies with *Candida guilliermondii* have shown that this endophyte yeast is able to penetrate the cuticle and colonize the apoplastic spaces without harming the cells, help to protect it against phytopathogens and loss of water and therefore extend its post-harvest life

Objectives

Evaluate changes on the surface of whole tomatoes inoculated with *Candida guilliermondii* Yeast Using AFM

Methods

Measurements of the surface topography were taken in the same area of the control tomatoes and the inoculated tomatoes at 0 (before inoculation), 2, 5, 8, 24, 48, and 72 hours after inoculation, the size of the scanned area was 50x50 μm^2. In addition to gauging the three-dimensional images of the surfaces, the changes in the roughness of the fruit in contact mode and in phase on non-contact mode were measured each time

Conclusions

The changes in fruit roughness were not perceived just 2 h after having been inoculated however, as time passed, the downward trend of this parameter was perceivable. Phase images taken in taping mode, made it possible to observe the changes in the mechanical properties on the surface of the fruit related to the adhered yeast and quantified by changes in phase. The variation in phase value compared with the control samples suggests that the biomechanics of the tomato's surface characteristics did change after having been inoculated with the yeast. The results would indicate that the surface of the fruit was homogenized after 72 hours due to the presence of a biofilm based on yeast that helps keep water inside the fruit

FEMS7-2713
Food Microbiology - Part II

POPULATION STRUCTURE OF HUMAN AND LIVESTOCK-ASSOCIATED STAPHYLOCOCCUS AUREUS ISOLATED FROM EAST AND WEST AFRICAN MILK PRODUCTS

C. Jans[1], A. Merz[2], S. Johler[2], M. Younan[3], S.A. Tanner[4], D.W.M. Kaindi[5], J. Wangoh[2], B. Bonfoh[6], L. Meile[1], T. Tasara[2]
[1]ETH Zurich,
Health Sciences and Technology- Institute of Food- Nutrition and Health- Laboratory of Food Biotechnology, Zurich, Switzerland
[2]University of Zurich, Institute of Food Safety and Hygiene, Zurich, Switzerland
[3]Vétérinaires Sans Frontières Germany, VSF, Nairobi, Kenya
[4]ETH Zurich, Health Sciences and Technology - Food Biotechnology, Zuerich, Switzerland
[5]University of Nairobi,
Department of Food Science- Nutrition and Technology- College of Agriculture and Veterinary Sciences, Nairobi, Kenya
[6]Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, CSRS,
Adiopodoumé Yopougon- Abidjan, Ivory Coast

Backgrounds

Staphylococcus aureus is a major pathogen responsible for food intoxication, human and animal diseases. *S. aureus* is a frequent contaminant of milk products in sub-Saharan Africa (SSA). SSA hospital-derived and human commensal *S. aureus* strains are well studied. However, the population structure and genetic features of foodborne *S. aureus* in SSA are largely unknown and hinder efforts to better identify possible staphylococcal food poisoning sources and mitigation strategies.

Objectives

The aim was to assess the population structure of milk-derived *S. aureus* isolates from Côte d'Ivoire, Kenya and Somalia in parallel with the presence of virulence and antibiotic resistance genes.

Methods

The population structure of *S. aureus* was assessed by *spa* typing, MLST, and DNA microarray analysis in comparison to the respective data repositories.

Conclusions

S. aureus isolates (n=70) obtained from milk samples of the three countries were assigned to 27 *spa* (7 new) and 23 (12 new) MLST sequence types. Genetically diverse human and livestock-associated clonal complexes (CCs) were observed among the milk-derived *S. aureus* isolates in this study. Isolates were predominantly assigned to CC5 (n=10) and CC30 (n=9). Virulence factors such as Panton-Valentine leukocidin, toxic shock syndrome toxin and enterotoxin encoding genes were predominantly observed among human-associated CCs. Penicillin, fosfomycin and tetracycline resistances were frequently detected. No methicillin resistance genes were detected. The population structure suggests that milk-derived *S. aureus* in SSA originates from human and animal sources alike. An overarching One Health approach to reduce *S. aureus* disease burdens through improving production processes, animal care and hygienic measures is thus recommended.

FEMS7-0786
Food Microbiology - Part II

PROBIOTIC PROPERTIES AND SAFETY ASSESSMENT OF BACILLUS STRAINS ISOLATED FROM KOREAN TRADITIONAL FERMENTED FOOD

H.L. Jeon[1], N.K. Lee[1], S.J. Yang[1], H.D. Paik[1]
[1]Konkuk University, Department of Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea

Backgrounds

The probiotics market is being expanded worldwide. Lactic acid bacteria (LAB) probiotics are usually studied and commercialized, whereas *Bacillus* strains are less popular as probiotics than LAB are.

Objectives

To isolate *Bacillus* strains from Korean traditional fermented food and to characterize their probiotic properties like acid and bile salt tolerance, adhesion ability, and enzymatic activity. In addition, the presence of toxic genes, antibiotic sensitivity and hemolysis were determined to evaluate safety.

Methods

Bacillus strains (p229, p230, and p231) were isolated from *cheonggukjang*, a fermented soybean food. According to 16S rRNA analysis, p229 was identified as *B. subtilis* and p230, and p231 were *B. licheniformis*. The spore cells of these strains showed high resistance to artificial gastric juice (pH 2.5, 0.3% pepsin) and bile salts (pH 7.0, 0.3 % oxgall). In addition, the spore cells of the strains had strong capacity for adhesion to HT-29 cells. The API zym kit was used to evaluate enzymatic activity; *Bacillus* strains did not produce β-glucuronidase, a carcinogenic enzyme. Antibiotic sensitivity was measured by a disk diffusion method and eight antibiotics (ampicillin, gentamicin, kanamycin, streptomycin, tetracycline, ciprofloxacin, chloramphenicol, and doxycycline) were tested. To evaluate safety, the presence of toxic genes was determined by a polymerase chain reaction and electrophoresis. Six kinds of toxic gene primers were used, and the *Bacillus* strains showed negative results for all these toxic genes. All strains showed no hemolysis on blood agar

Conclusions

The *Bacillus* strains isolated from *cheonggukjang* have a potential as probiotics for functional foods

FEMS7-1474
Food Microbiology - Part II

SCREENING AND CHARACTERIZATION OF LACTIC ACID BACTERIA AS POTENTIAL PROBIOTICS ISOLATED FROM KIMCHI

H.L. Jeon[1], S.H. Son[1], N.K. Lee[1], Y.J. Kim[2], H.D. Paik[1]
[1]Konkuk University, Department of Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea
[2]Korean Cultrue Center of Microorganisms, Korean Cultrue Center of Microorganisms, Seoul, Republic of Korea

Backgrounds

Lactic acid bacteria (LAB) as probiotics are being studied, and the probiotics market is expanding worldwide.

Objectives

To isolate LAB strains from a Korean traditional plant fermented food, kimchi, and to screen them for potential probiotics.

Methods

For isolation of strains, selective media were use: MRS, PES (phenyl ethyl alcohol sucrose), and LBS (*Lactobacillus* selective) agar. Thirty isolates were obtained. At first screening for acid tolerance, 5 strains showed strong acid tolerance (over 70%) to pH 2.5 MRS broth for 24 h. The survival rate of 2 strains (KCCM 200060 and KCCM 200070) in artificial gastric juice (pH 2.5, 0.3% pepsin for 3 h) and bile salt (pH 7.0, 0.3% oxgall for 24 h) was over 80%. For identification of strains, 16S rRNA analysis was used. KCCM 200060 was identified as *Lactobacillus fermentum* and KCCM 200070 as *Pediococcus damnosus*. Enzymatic activity was determined using the API zym kit. KCCM 200060 did not produce β-glucuronidase, a carcinogenic enzyme, but KCCM 200070 did. The cytotoxicity of these bacteria to normal cells (MRC-5, human lung fibroblast cell) was measured by an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, and KCCM 200060 had no cytotoxicity toward MRC-5 cells. KCCM 200060 showed strong capacity for adhesion to human colon adenocarcinoma cells (HT-29). Antibiotic sensitivity was determined using a disk diffusion assay. KCCM 200060 was resistant to 4 of 8 antibiotics; gentamicin, kanamycin, streptomycin, and ciprofloxacin.

Conclusions

L. fermentum KCCM 200060 has a potential as a probiotic starter.

FEMS7-0522
Food Microbiology - Part II

GENOMIC INSIGHTS OF STAPHYLOCOCCUS EQUORUM AS A STARTER CANDIDATE FOR HIGH-SALT-FERMENTED FOODS

D.W. Jeong[1], S. Heo[2], H. Lee[2], K. Jeong[2], J.H. Lee[2]
[1]Dongduk Women's University, Department of Food and Nutrition, Seoul, Republic of Korea
[2]Kyonggi University, Department of Food Science and Biotechnology, Suwon, Republic of Korea

Backgrounds

Staphylococcus equorum has been identified as a predominant bacterial species in jeotgal, a traditional Korean high-salt-fermented seafood. Little has been known about its contribution and metabolic process in the fermentation of jeotgal.

Objectives

The objectives of the current research are to understand roles of the *S. equorum* in jeotgal fermentation.

Methods

The gene repertoire contributing to the lifestyle of the jeotgal starter candidate *S. equorum* KS1039 was deduced from its complete genome sequence.

Conclusions

S. equorum KS1039 genome is equipped with several genes for fermentations and color developments and can confer the capability to catabolize cellobiose, maltose, mannose, mannitol, sucrose, and trehalose. *S. equorum* KS1039 can produce flavor compounds including acetoin, butanediol, and branched chain fatty acids. While, *S. equorum* KS1039 does not possess decarboxylase genes, which can contribute to produce biogenic amines. Uniquely, *S. equorum* KS1039 possesses the genes encoding bacteriocin and CRISPR/Cas system. This genome analysis of *S. equorum* KS1039 revealed genetic insight into the safety and efficacy of this strain as a candidate starter culture for high-salt food fermentation.

FEMS7-0140
Food Microbiology - Part II

PCR AND ELISA FOR STAPHYTAPHYLOCOCCAL ENTEROTOXINS AND DETECTION OF SOME EXOTOXINS FROM STAPHYLOCOCCUS SPP. STRAINS BY PCR

S. Kahya Demirbilek[1], *Ö. Yılmaz*[1]
[1]*Veterinary Faculty, Microbiology, bursa, Turkey*

Backgrounds

A PCR assay was used to investigate the presence of important exotoxins (enterotoxins, *eta-etb*, and *tst*) in CNS and CPS strains isolated from ground beef and lamb meat for the first time in Turkey.**Objectives**The aims of this study were to determine the existence of some staphylococcal enterotoxin (SE) (*sea, seb, sec, sed,* and *see*) proteins and genes in coagulase-positive staphylococci (CPS) by ELISA and PCR and to assess SE-like toxin (SEI) (*seg, seh, sei, sej, sem, sen,* and *seo*), exfoliative toxin (*eta* and *etb*), toxic shock syndrome toxin-1 *(tst*) and 16S rRNA genes in 11 different *Staphylococcus* strains [90 CPS and 118 coagulase-negative staphylococci (CNS)] isolated from 250 ground meat samples by either monoplex or multiplex PCR.**Methods**

ELISA

SEs from 90 CPS isolates were assessed using commercially available kits (Ridascreen SET A, B, C, D, E, R-biopharm, Germany, Art no: R1101).

Detection of SE, SEI, *eta-etb*, *tst* and 16S rRNA genes by PCR

Six different mixtures were prepared for monoplex and multiplex PCR analyses**Conclusions**

SEs were identified in 36 (40%) out of 90 CPS isolates by both ELISA and PCR with the following distribution: *sea* was identified in 7 (7.7%), *seb* in 5 (5.5%), *sec* in 3 (3.3%), *sed* in 4 (4.4%), and *see* in 17 (18.8%). In addition, a total of 90 CPS and 118 CNS isolates were investigated for the presence of 11 SE, SEI, *eta-etb, tst,* and 16S rRNA genes. Overall, 145 (69.7%) of the *Staphylococcus* spp. isolates tested positive for one or more toxin genes. These results indicate that CNS may play an important role in food poisoning and that SEI toxins must be investigated in greater detail in future studies of both CPS and CNS.

FEMS7-2775
Food Microbiology - Part II

POPULATION STRUCTURE AND PHYLOGENETIC ANALYSIS OF STREPTOCOCCUS INFANTARIUS SUBSP. INFANTARIUS COLLECTED FROM HUMAN FECAL MATERIAL IN KENYA IN COMPARISON TO DAIRY, COMMENSAL AND PATHOGENIC ISOLATES

D.W.M. Kaindi[1], W. Kogi-Makau[1], G.N. Lule[2], B. Kreikemeyer[3], P. Renault[4], B. Bonfoh[5], E. Schelling[6], J. Zinsstag[6], N. Otaru[7], T. Schmid[7], C. Lacroix[7], L. Meile[7], J. Hattendorf[6], C. Jans[7]
[1]University of Nairobi,
Department of Food Science- Nutrition and Technology- College of Agriculture and Veterinary Scienc es, Nairobi, Kenya
[2]University of Nairobi, School of Medicine, Nairobi, Kenya
[3]University Medical Centre Rostock, Institute of Medical Microbiology- Virology- and Hygiene, Rostock, Germany
[4]Institut National de la Recherche Agronomique, Micalis Institute, Jouy-en-Josas, France
[5]Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, CSRS,
Adiopodoumé Yopougon- Abidjan, Ivory Coast
[6]Swiss Tropical and Public Health Institute- University of Basel,
Department of Epidemiology and Public Health, Basel, Switzerland
[7]ETH Zurich,
Health Sciences and Technology- Institute of Food- Nutrition and Health- Laboratory of Food Biotechn ology, Zurich, Switzerland

Backgrounds

High amounts of *Streptococcus infantarius* subsp. *infantarius* (*Sii*) are consumed in traditional fermented dairy products in East and West Africa. However, the population structure of *Sii* of human and dairy origin and their health risks as *Streptococcus bovis/Streptococcus equinus* complex (SBSEC) members has not been elucidated.

Objectives

To elucidate the population structure of *Sii*/SBSEC obtained from clinical specimen and to compare the isolates with globally relevant *Sii*/SBSEC strains of dairy, commensal and potentially pathogenic lineages as a proxy for assessing risk to human health.

Methods

Sii/SBSEC strains (n=40) from rectal swabs of persons undergoing colonoscopy at Kenyatta National Hospital, Nairobi, Kenya were analyzed by SBSEC-MLST for phylogeny and screened for dairy adaptation marker genes. For comparison, 43 other *Sii* strains were included from Kenyan infant faeces (n=8), dairy products of East (n=20) and West Africa (n=9), human blood (n=3), animal source (n=1), Grana cheese (n=1) and fermented maize (n=1).

Conclusions

The 40 SBSEC strains from rectal swabs comprised *Sii* (n=33), non-*Sii* SBSEC pathogens (n=6) and *Streptococcus macedonicus* (n=1). One of these 33 *Sii* clustered with presumed pathogenic *Sii* strains obtained from blood cultures. The remaining 32 rectal swab *Sii* clustered with *Sii* from human faeces from Kenyan infants and West African dairy products, clearly separated from East Africa dairy *Sii*. In the African context, this suggests a complex exchange of *Sii* across ecologic niches to feature a shared *Sii* human commensal and West African dairy clade and the evolution of a specific East African *Sii* dairy lineage possibly relevant for local dairy fermentation.

FEMS7-1610
Food Microbiology - Part II

PREVALENCE AND POPULATIONS OF PATHOGENIC BACTERIA IN BRAZILIAN ARTISANAL CHEESES

B.A. Kamimura[1], L. Pereira Margalho[1], V. Ortiz Alvarenga[1], L. do Prado Silva[1], R. Peres Brexó[1], A. de Souza Sant'Ana[1]
[1]State University of Campinas, Food Science, Campinas, Brazil

Backgrounds

The production of artisanal cheeses (AC) is characterized by tradition and culture of producing regions. Every cheese has unique sensory characteristics typical of its region.

Objectives

This study aimed to determine the incidence and concentration of *Listeria monocytogenes*, *Salmonella* and *Staphylococcus* spp. in Brazilian AC.

Methods

We analyzed 582 samples of Brazilian AC, divided according to their production region: North (Marajó; n=8); Northeast (Curd cheese; n=78) and (Butter cheese; n=23); Central (Caipira cheese; n=108); Southeast (Araxá; n= 56), (Campo das Vertentes; n= 54), (Cerrado; n= 54), (Serra da Canastra; n= 48) and (Serro; n= 50) and South cheeses (Colonial; n= 55) and (Serrano; n= 48). Detection and enumeration of *L. monocytogenes* was performed according to ISO 11290-1 and ISO 11290-2. Detection of *Salmonella* based on ISO 6579: 2002, while for enumeration ISO 6579-2: 2012 was used. *Staphylococcus* spp. was performed according to ISO 6888-3: 2003 and ISO 6888-1: 1999 for detection and enumeration. Identification of *L. monocytogenes* and *Staphylococcus* spp. were performed by biochemical tests and real-time PCR (RT-PCR) with amplification of listeriolysin gene (*hlyA*) and *femA* gene.

Conclusions

L. monocytogenes and *Salmonella* were not detected in any samples analyzed. The highest concentration of *Staphylococcus* spp. was 6.85 ± 1.27 log CFU.g^{-1} in Campo das Vertentes cheeses. The data obtained in this large survey indicates that *Staphylococcus* spp. seems to be the main bacterial genus present in cheeses that may impact on their safety and public health. Further studies are being performed to characterize the pathogenic potential of *Staphylococcus* strains isolated from AC.

FEMS7-2660
Food Microbiology - Part II

RETROSPECTIVE STUDY OF LISTERIA MONOCYTOGENES ORIGINATING FROM FOODS AND HUMANS IN OUTBREAK INVESTIGATION

R. Karpíšková[1], *Z. Tomáštíková*[1], *M. Zobaníková*[1], *T. Gelbíčová*[1]
[1]*Veterinary Research Institute, Department of Bacteriology, Brno, Czech Republic*

Backgrounds

L. monocytogenes is an emerging foodborne pathogen, causative agent of listeriosis. Ready-to-eat foods are often the source of listeria infections especially those with long shelf life that support the bacterial growth.

Objectives

The aim of the study was to identify cases with possible epidemic context by typing of *L. monocytogenes* isolated from food and human.

Methods

Altogether 116 human and 141 ready-to-eat food isolates of *L. monocytogenes* were collected in years 2013 – 2016. The isolates were characterized by serotyping, PCR clonogrouping, and macrorestriction analysis. Selected strains were analysed using the core genome multilocus sequence typing (cgMLST).

Conclusions

In the Czech Republic (CZ) serotype 1/2a, clonal complex CC8 belongs to the most frequent ones in both the human population and food isolates. Since 2013 an increased number of listeriosis caused by strains of serotype 1/2a, pulsotype 810, sequence type ST8 (22 cases from one locality of CZ) and strains of serotype 4b, pulsotype 203, ST6 (18 cases from different localities of CZ) were observed. The typing of *L. monocytogenes* from food enabled to confirm the vehicle (turkey meat delicatessen) of local outbreak in the Moravian-Silesian Region. On the other hand only one food strain of serotype 4b, pulsotype 203 was detected from pork ham. With respect to the occurrence of geographically separated cases and rare findings in foods it was not possible to identify the source of infection.

Acknowledgements: This study was carried out with the financial support of projects AZV 16-31488A.

FEMS7-2744
Food Microbiology - Part II

ENTEROTOXIGENIC STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM FOODSTUFF IN THE CZECH REPUBLIC

I. Koláčková[1], M. Zobaníková[1], H.A. Tegegne[2], R. Karpíšková[1]
[1]Veterinary Research Institute, bacteriology, Brno, Czech Republic
[2]University of Veterinary and Pharmaceutical Sciences Brno, Milk Hygiene and Technology, Brno, Czech Republic

Backgrounds

The importance of *Staphylococcus aureus* in food is assessed by the potential of enterotoxins production and their ability to cause foodborne diseases - staphylococcal food poisoning. Mixed food, dairy and meat products, poultry and pork meat are frequently reported as source of such foodborne outbreaks.

Objectives

The study was conducted to find out the relationship between the potential of enterotoxins production and the origin of *S. aureus* strains.

Methods

The collection of 341 *S. aureus* strains were isolated from meat and liver (pork, beef, and poultry), milk (cow, goat and sheep) and milk products, food of plant origin, delicatessen and desserts. All strains were screened for the presence of five classical enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*) and 4 enterotoxin-like genes (*seg*, *seh*, *sei, sej)* by using PCR method.

Conclusions

At least one gene was found in 54% of isolates. Classical enterotoxins were found in 35% of strains isolated across the whole spectrum of monitored food. Out of enterotoxins-like genes, *seg* and *sei* were most often positive in almost all types of food but mainly in poultry (43%). Findings of *seh* gene were strongly connected with pork meat and liver (71%). Due to the thermostability of staphylococcal enterotoxins, such contaminated pork could serve as a vehicle for intoxication. Nowadays laboratory testing for the presence of enterotoxins in food may produce false negative results due to the commonly used methods, which cannot detect the new types of toxin.
Acknowledgements: This study was carried out with financial support of QJ1210284, QJ1510216.

FEMS7-3036
Food Microbiology - Part II

THE ABILITY OF LACTIC ACID BACTERIA ENTERING INTO A CONSORTIUM TY SYNTHESIZE EXOPOLYSACHARIDES

A. Sadanov[1], G. Jakibaeva[1], A. Molzhigitova[1]
[1]Institute of microbiology and virology, GS MES RK, Almaty, Kazakhstan

Backgrounds

A significant place among microorganisms capable of producing exopolysaccharides is occupied by lactobacillus bacteria of the genus Lactobacillus.

Lactobacilli have a great potential for the synthesis of exopolysaccharides, but the functions of these biopolymers are not fully understood. To form a concept of the influence of exopolysaccharides of lactic acid *bacteria* on physiological reactions in the animal body, it is necessary to accumulate data on the chemical structure, physical and biological properties of polysaccharides of different species and strains. Check the ability of lactic acid bacteria entering into the consortium ty synthesize polysaccharides.

Objectives

The objects of the study were collection milk acid bacteria: *Lactobacillus plantarum* 53H, *Lactobacillus plantarum* 22,*Lactobacillus plantarum* 2*, Lactobacillus cellobiosus* 20,*Lactobacillus acidophilus* 27*W, Lactobacillus curvatus* 18д, *Lactobacillus casei* 139, *Lactobacillus casei* 173a, *Lactobacillus salivarius* 8д, *Lactobacillus fermentium* 27 and milk acid bacteria: *Lactococcus lactis* K-1, *Streptococcus thermophilus* K-2, *Lactobacterium bulgaricus* K-3, *Lactococcus lactis* 8, *Streptococcus lactis* 6, *Saccharomyces lactis* 14c , *Saccharomyces lactis* 19, included in consortia.

Methods

Microbiological methods of research were used in the work.

Conclusions

Testing the ability of collection strains to synthesize exopolysaccharides showed that of the tested 10 cultures, only three lactic cultures (Lactobacillus casei 139, Lactobacillus plantarum No. 2, Lactobacillus cellobiosus No. 20) synthesized exopolysaccharides. Moreover, in the culture of Lactobacillus plantarum No.2 basically, all the colonies remained white and only a few colonies acquired a pink color. This indicates the heterogeneity of the population in terms of biochemical characteristics. The results obtained can be used in the food industry.

FEMS7-2443
Food Microbiology - Part II

COMPARISON OF THREE TYPES JUICY, UNDER COOKED AND COOKED PERFECTLY STEAKS IN TERMS OF MICROBIAL LOAD

Z. Mirzaei[1], N. Khakipour[2]
[1]M.Sc graduated student of Food Science & Technology- Department of Agriculture- Savadkooh Bran ch- Islamic Azad University- Savadkooh- Iran, ., Savadkooh, Iran
[2]Department of Agriculture- Savadkooh Branch- Islamic Azad University, ., Savadkooh, Iran

Backgrounds

Due to population increases, the need for resources protein becomes more. Thus providing enough food has always been regarded with attention to health. On the other hand, given that *Escherichia coli* and Salmonella.sp that cause food-borne illnesses. The world's hundreds of millions of people are suffering from diseases transmitted through food and water.

Objectives

The preparation of food without pollution to the bacteria in the food manufacturing industry has been a constant concern.

Methods

In this study 12 samples of raw meat, juicy steak, cooked medium were examined in term microbial contamination to total count, E. coli and Salmonella in accordance with standard guidelines to numbers 1-5272, 2946 and 1810 respectively.

Conclusions

The results showed that all samples of the bacterial contamination was detected in standard and disposable. According to tests carried out by microbial all raw and cooked meat samples, in three juicy, medium and perfectly cooked in terms of bacterial contamination was less than the standard limit as well as was not observed significant differences between the three samples juicy steak, under cooked and cooked perfectly ($p>0.05$). Therefore result was considered consumable. That their consumption, there is not concern for the consumer. By examining the two parameters of time and temperature on the bacterial contamination of the tested meat steaks, the results of microbiological tests showed that Bacterial contamination, the samples were perfectly cooked steak compared to the state is less than half cooked and juicy. It is recommended the meat perfectly cooked steaks with full thermal process, should be used.

FEMS7-2842
Food Microbiology - Part II

INHIBITION OF LISTERIA MONOCYTOGENES IN MINCED FISH BY USE OF DIFFERENT ALGAE EXTRACTS

M. Khezri[1], M. Rezaei[1], S. Rabiei[1], M. Zolfaghari[1], G. Pesavento[2]
[1]Tarbiat Modares University, Department of Seafood processing- Faculty of Marine Sciences, Noor, Iran
[2]University of Florence, Health Sciences Department- Applied Microbiology Laboratory, Florence, Italy

Backgrounds

Listeria monocytogenes, causative of listeriosis, is one of the most virulent food-borne pathogens worldwide which is frequently reported in seafood products.

Objectives

The aim of this study was to evaluate the inhibitory activity of different algae extracts against *Listeria monocytogenes* in minced fish.

Methods

In this study, Methanol, acetone and chloroform extracts of seven marine algae including *Hypnea hamulosa, Gracilaria corticata, Sargassum angustifolium, Colpomenia sinuosa, Dictyota dichotoma, Chaetomorpha* sp. and *Enteromorpha intestinalis* were investigated for their antibacterial activity against *Listeria monocytogenes* using paper disc diffusion method (Experiment 1). The dried methanol extract of *E. intestinalis* was also applied in minced fish at 0.05%, 0.075%, and 0.1% (w/w) (experiment 2) and the numbers of *Listeria monocytogenes* were counted during storage in refrigerator.

Conclusions

The highest inhibitory activity was shown by methanol extract of *E. intestinalis*. The main identified bioactive component by GC/MS analysis were Cycloheptasiloxane, tetradecamethyl (19.76%), (Z, E)-3,13-octadecadien-1-ol (14.98%), Cyclohexasiloxane, dodecamethyl (12.17%), cis-13,16-Docasadienoic acid, methyl ester (8.80%), 13-Octadecenal, (Z) (8.61%), Cyclodecasiloxane, eicosamethyl (7.58%) and 9-Octadecenoic acid (Z) (5.62%). Methanol extract of *E. intestinalis* at 0.05%, 0.075%, and 0.1% (w/w) reduced respectively the *L. monocytogenes* viable count by 3.23, 3.32 and 3.47 log $cfu.g^{-1}$ after 10 days of storage ($p<0.05$). In conclusion, the methanol extract of *E. intestinalis* showed favorable inhibitory effect against *L. monocytogenes,* which could be considered in the food and pharmaceutical industry.

FEMS7-2847
Food Microbiology - Part II

THE APPEARANCE OF VIABLE BUT NON CULTURABLE STATE IN LISTERIA MONOCYTOGENES DURING FISH BRINING

M. Zolfaghari[1], M. Rezaei[1], A. Mohabbati Mobarez[2], H. Hosseini[3], M. Khezri[1]
[1]Tarbiat Modares University, Department of Seafood processing- Faculty of Marine Sciences, Noor, Iran
[2]Tarbiat Modares University, Department of Bacteriology- School of Medical Sciences, Tehran, Iran
[3]Shahid Beheshti University of Medical Sciences,
Department of Food Science and Technology- Faculty of Nutrition Sciences and Food Technology, Tehran, Iran

Backgrounds

Listeria monocytogenes is a foodborne pathogen which has high resistance for unfavorable conditions. This pathogen is able to entering Viable but non culturable (VBNC) state in harsh conditions.

Objectives

The present study was aimed to consider the possibility of entering of *Listeria monocytogenes* in to VBNC state in heavy brine which used to produce brined fish.

Methods

L. monocytogenes was monitored in three treatments during time: brine containing 30% salt (ES30), brine containing 10% salt (ES10) and starvation condition (DE). For considering alive cells method of gene expression of 16S rRNA was used.

Conclusions

The obtained results showed that this pathogen in ES30 and DE treatments after three days enter in to VBNC state. According to obtained results ES10 treatment entering in to VBNC state on 5 days after inoculation. The results showed that there is the possibility of *L. monocytogenes* to enter into VBNC state during brined and smoked fish or every other brined food product as well as the defect of standard detection methods.

FEMS7-0137
Food Microbiology - Part II

IMPROVED CHARACTERISTICS OF KIMCHI AFTER USING LEUCONOSTOC STRAINS AS A STARTER AND OPTIMIZED CULTIVATION OF THE LEUCONOSTOC STRAIN

B.C. Kim[1], J.H. Lee[1], S.C. Song[1], P. Yong-Ha[1]
[1]ProBionic Corp., Research division, Jeonju-si, Republic of Korea

Backgrounds

Kimchi, fermented vegetable side dishes in Korea, is made with a variety of ingredients, mainly with Korean cabbage and radish, with or without Jeotgal, a salty fermented fish. After mixing all ingredients, the vegetables in Kimchi are fermented by lactic acid bacteria (LAB) during its storage. The taste and storage period of Kimchi is mainly determined by the quality of ingredients and fermentation. During fermentation process, the pH is decreased according to the increase of LAB, and the frequency of some LAB such as *Weissella* sp. *Lactobacillus* sp. and *Leuconostoc* sp. are increased. Among diverse LAB, some hetero fermentative LAB especially *Leuconostoc* spp. mainly influence the taste and flavor of Kimchi.

Objectives

In this study, the improved characteristics of Kimchi after using *Leuconostoc* strains as Kimchi starter were surveyed for the production of high-quality Kimchi. In addition, the growth of *Leuconostoc* strain at diverse culture conditions was observed for commercial production of Kimchi starter.

Methods

Korean cabbage Kimchi was made by a standard recipe for Kimchi. In the initial mixing process, *Leuconostoc* strains used as Kimchi starter were mixed with other ingredients without Korean cabbage. Then the mixture was applied to Korean cabbage. The taste and storage time of Kimchi were measured for the understating of the effects of Kimchi starter. The growth characteristics of *Leuconostoc* strains were observed according to the basic bacteria cultivation protocol for the production of Kimchi starter.

Conclusions

Application of *Leuconostoc* strains as a starter to Korean cabbage enhanced the taste and the storage period of Kimchi. It seems the addition of starter influenced the acidity of Kimchi at initial fermentation stage. Similarly to other LAB, the cell number of *Leuconostoc* strain for Kimchi starter increased at its optimum growth pH range, and three times increase in cell number was observed at pH regulated fermentor culture.

FEMS7-0322
Food Microbiology - Part II

COMPARATIVE GENOME ANALYSIS OF VIBRIO PARAHAEMOLYTICUS REVEALED GENETIC CHARACTERIZATION OF V. PARAHAEMOLYTICUS FORC_004

S. Ahn[1], H. Kim[2]
[1]Seoul National University, Interdisciplinary Program in Bioinformatics, Seoul, Republic of Korea
[2]Seoul National University, Department of Food and Animal Biotechnology, Seoul, Republic of Korea

Backgrounds

Vibrio parahaemolyticus is one of the significant pathogenic agents and capable of causing seafood poisoning in humans in case of consumption of contaminated raw or undercooked seafood. Until recently, *V. parahaemolyticus* pathogenicity of which has been characterized by the presence of *tdh*, *trh* and type three secretion systems(TTSSs). Here, we completed genome sequence of suspected pathogen causing food borne illness *V. parahaemolyticus* FORC_004 without *tdh* and *trh.*

Objectives

V. parahaemolyticus FORC_004, which is suspected pathogen related food poisoning was sequenced to characterize of its genome and investigate the pathogenicity.

Methods

The *V. parahaemolyticus* FORC_004 was isolated from aquarium water in Korea. Whole genome sequencing was performed using Pacbio RS II. Subsequently, reads from sequencing platform were assembled using SMRT portal system with HGAP assembly-3 algorithm. The bioinformatic analysis was performed using various programs such as RAST, Prokka, BASys, JSpecies, WebMGA, BLAST and Roary.

Conclusions

The *V. parahaemolyticus* FORC_004 is composed of two circular chromosomes and one plasmid which are 3,348,850 bps, 1,758,762 bps and 64,049 bps with a GC content of 45.48%. We identified 4,723 protein coding genes in FORC_004 chromosomes and plasmid and among these, 3679 genes assigned to COGs. The FORC_004 strain contains various virulence genes such as *toxR*, collagenase, and TTSS-1 related genes without *tdh* and *trh*. The ANI analysis revealed that FORC_004 strain clustered with BB22OP, RIMD2210633, and CDC_K4557 which are reported virulence strains. Furthermore, we conducted pan-genome analysis. As a result, antitoxin *chpS* and Type IV secretion system protein VirB3, VirB4, and VirB11 were found in only FORC_004.

FEMS7-0958
Food Microbiology - Part II

COMPARATIVE TRANSCRIPTOME ANALYSIS OF CLINICAL ISOLATES OF SALMONELLA ENTERICA ENTERITIDIS IN CONTACT WITH FRESH CABBAGE AND LETTUCE

S. Hong[1], M. Shim[1], B.R. Kim[1], J. Shin[1], J. Lee[1], R.B. Guevarra[1], J.H. Lee[2], H. Yoon[3], S.I. Kim[3], H.B. Kim[1]
[1]Dankook University, Animal Resources Science, Cheonan, Republic of Korea
[2]Kyung Hee University, Department of Food Science and Biotechnology, Yongin, Republic of Korea
[3]Ajou University, Department of Applied Chemistry and Biological Engineering, Suwon, Republic of Korea

Backgrounds

Salmonella enterica is one of the leading causes of food borne disease in the world. Recently, many cases of Salmonellosis outbreaks are caused by *Salmonella*-contaminated fresh vegetables, and *S. enterica* serotype Enteritis is one of the most common *Salmonella* serotypes associated with food borne diseases.

Objectives

The aim of this study was to understand the systemic gene regulations of *S. enterica* serotype Enteritis in contact with fresh cabbage and lettuce.

Methods

S. enterica serotype Enteritis isolated from the feces of infected patients in South Korea were inoculated onto cabbage and lettuce leaves in a minimal medium broth for 2.5 hours, respectively. The bacterial mRNA was sequenced using MiSeq RNA-Seq chemistry. All the experiments were duplicated.

Conclusions

The nitrate-responsive two-component system NarX-NarL genes were significantly up-regulated in *S. enterica* serotype Enteritis in contact with fresh cabbage and lettuce.Also, genes associated with flagella assembly, chemotaxis, and T3SS were significantly up-regulated in both cases. High concentration of nitrate is often found on cabbage and lettuce because of nitrate fertilizer used to grow vegetables, thus up-regulation of the nitrate-responsive two-component system NarX-NarL genes can be beneficial to bacteria growing and colonizing fresh vegetables. It has also been suggested that up-regulations of genes associated with motility, chemotaxis, and T3SS are required for bacteria to colonize vegetables through open stomata. In conclusion, results from this study suggest that the nitrate-responsive two-component system NarX-NarL and genes associated with motility, chemotaxis, and T3SS of *S. enterica* can be potential targets for genetic regulations to reduce *Salmonella* colonization in vegetables.

FEMS7-0669
Food Microbiology - Part II

PAN-GENOME AND METATRANSCRIPTOME ANALYSES REVEAL INSIGHTS INTO THE GENOMIC AND METABOLIC FEATURES OF LEUCONOSTOC MESENTEROIDES DURING KIMCHI FERMENTATION

K.H. Kim[1], B.H. Chun[1], S.E. Jeong[1], C.O. Jeon[1]
[1]Chung-Ang university, life science, Seoul, Republic of Korea

Backgrounds

Leuconostoc (*Leu*) *mesenteroides*, a typical heterofermentative lactic acid bacterium, is generally predominant during kimchi fermentation. However, genomic and metabolic features of *Leu. mesenteroides* have not been extensively explored yet.

Objectives

To investigate genomic features and metabolic properties during kimchi fermentation of *Leu. mesenteroides*, its comprehensive pan-genome and metatranscriptome analyses were performed.

Methods

High quality genomes of *Leu. mesenteroides* were retrieved from GenBank and pan-genome and COG analyses were performed for the estimation of genomic and functional properties. In addition, for the analysis of metabolic features of *Leu. mesenteroides*, all protein sequences were mapped into KEGG pathways and carbon metabolic pathways of *Leu. mesenteroides* were reconstructed. Finally, metatranscriptomic analysis of *Leu. mesenteroides* against the carbon metabolic pathways was performed during kimchi fermentation.

Conclusions

Pan-genomes of eighteen *Leu. mesenteroides* strains consisted of 3,948 pan-genes and 825 core-genes. Phylogenetic analysis based on core-genes showed that *Leu. mesenteroides* strains were differentiated into five different lineages, which was a little different from that based on 16S rRNA gene sequences. Functional properties of *Leu. mesenteroides* by COG analysis were relatively similar with those of other *Leuconostoc* species and *Weissella* species, while they were a little different with those of *Fructobacillus* species. KEGG analysis showed that *Leu. mesenteroides* have abundant carbohydrate metabolic genes and sugar phosphotransferase systems. Metabolic pathway reconstruction suggested that *Leu. mesenteroides* may various metabolites including lactic acid, acetate, ethanol, CO_2, mannitol, 2,3-butanediol, acetoin, and dextran as fermentation products. In addition, metatranscriptomic analysis was performed to investigate metabolic properties of *Leu. mesenteroides* during kimchi fermentation.

FEMS7-1346
Food Microbiology - Part II

NEW KNOWLEDGE ABOUT AN OLD ENEMY: THE HEAT RESISTANT SALMONELLA SENFTENBERG 775W

S. Knøchel[1], *E.J. Boll*[2]
[1]*University of Copenhagen, Dept. Food Science, Frederiksberg, Denmark*
[2]*Statens Serum Institut, Department of Microbiology and Infection Control, Copenhagen, Denmark*

Backgrounds

Salmonella is a common cause of food poisoning and much effort has gone into predicting the heat inactivation of different strains. More than 50 years ago, a particular strain, *Salmonella* senftenberg 775W (=ATCC 43845), originally isolated from dried egg in 1946, was observed to be extremely heat resistant. Several researchers have reported D_{60} -values of this strain to be in the order of 10 times longer compared with strains of "normal" heat resistance. Although the strain has since been used in numerous inactivation studies, the reason for this unusual resistance has never been elucidated and other enhanced resistance phenotypes of this strain have not been reported

Objectives

The aim was to investigate the possible involvement of a recently described locus of heat resistance (LHR) found in some isolates of gram-negative bacteria in the heat resistance of *Salmonella* senftenberg 775W

Methods

The published genome of *Salmonella* senftenberg 775W was used to identify two LHR in this strain. The *clpK* genes as well as the LHR1 and LHR2 clusters of *Salmonella* Senftenberg 775W were subsequently deleted by allelic exchange and inactivation studies were performed

Conclusions

Salmonella senftenberg 775W harbors two variants of a locus of heat resistance (LHR) genes. The heat resistant phenotype of 775W is attributable to the two LHRs and, more specifically, to the LHR-encoded ClpK belonging to the family of Clp ATPases with chaperone activity. Notably, the wild type also exhibited another non-thermal stress resistant phenotype compared with the mutants indicating that LHR-containing strains have an enhanced survival potential in different settings.

FEMS7-3204
Food Microbiology - Part II

VANCOMYCIN RESISTANCE IN LACTOBACILLUS SP. IS AN EVOLUTIONARY TRAIT AND NOT DEPENDENT ON FERMENTATIVE PATTERNS

G.M. Knudsen[1], *B. Stuer-Lauridsen*[1], *Y. Agersø*[1], *E.J. Boll*[1], *A. Wind*[1]
[1]*Chr. Hansen, Identification, Hørsholm, Denmark*

Backgrounds

Some species of *Lactobacillus* such as *L. plantarum* are vancomycin resistant, whereas others such as *L. acidophilus* are sensitive. For food and feed cultures, the European Food Safety Authority (EFSA) set cut-off values to identify antimicrobial resistant strains as they may contain transmissible antibiotic resistance. For the heterogeneous group of *Lactobacillus* sp. the cut-off values are mainly based on fermentative status i.e. homofermentative species being sensitive and heterofermentatives being resistant. However, the homofermentative *L. mali* is vancomycin resistant.

Objectives

The aim of this study was to elucidate the vancomycin resistance pattern of the *Lactobacillus* genus.

Methods

A BlastX search of the genome of *L. mali* LBA-20315 against the ResFinder database was performed, but no hits with more than 50% identity (E value > 1.9×10^{-54}) to vancomycin resistance genes were detected. To investigate the vancomycin resistance pattern in a broader context, the susceptibility towards vancomycin of 25 species (N= 398) was investigated. In addition to *L. mali,* vancomycin resistance was observed within the homofermentative species *salivarius* and *farciminis*. The presence of genes involved in vancomycin resistance i.e. *ldh*, *aad*, *lar* and *ddl* was investigated in 11 species and no correlation with vancomycin resistance was observed. However, phenotypic vancomycin resistance correlated with different alleles of *ddl* encoding a D-alanine-D-alanine ligase also referred to as *vanA*.

Conclusions

Thus, this study indicates that vancomycin resistance is an evolutionary trait depending of variation within the *ddl* gene causing intrinsic resistance and is independent of fermentative status. Sequencing of further species is ongoing to support these conclusions.

FEMS7-2855
Food Microbiology - Part II

BIOFILM FORMATION BY LISTERIA SPP., LACTOBACILLUS SPP. AND PSEUDOMONAS SPP. AND INACTIVATION OF CELLS IN SINGLE-SPECIES BIOFILMS

A. Kocot[1], M. Olszewska[2], Ł. Łaniewska-Trokenheim[2]
[1]University of Warmia and Mazury in Olsztyn,
Faculty of Food Science- Chair of Industrial and Food Microbioogy, Olsztyn, Poland
[2]University of Warmia and Mazury in Olsztyn,
Faculty of Food Science- Chair of Industrial and Food Microbiology, Olsztyn, Poland

Backgrounds

The biofilm is the most common mode of bacterial existence in various environments, including food processing environments. In the food processing context, the biofilm is considered as a serious hygienic challenge and a greater risk factor of bacterial transmission. Therefore studies concerning formation dynamics and inactivation of biofilms are crucially important parts of food safety.

Objectives

1. Estimation of the ability of *Listeria* spp., *Lactobacillus* spp. and *Pseudomonas* spp. to form biofilms.
2. Examination of the formation dynamics of single-species biofilms.
3. Evaluation of the biofilm resistance to disinfectants used in the food industry.

Methods

The ability to form biofilms was studied using the microtiter plate assay with crystal violet (CV) staining. The biofilm formation dynamics in 24-h intervals and cell inactivation after 72-h incubation were examined by plate counting and fluorescence staining with calcein-AM and TOTO-1, which gave an insight into changes in the bacterial culturability and cell membrane integrityat the single cell level.

Conclusions

Listeria spp., *Lactobacillus* spp. and *Pseudomonas* spp. showed differences in the ability to form biofilms and the biofilm formation dynamics over 72-h incubation. *Listeria* spp. and*Lactobacillus* spp. grew well and to higher cell counts whereas *Pseudomonas* spp. grew slowly and to lower cell counts. Although all disinfectants used in this study reduced cell numbers in biofilms to some extent, their persistence may still pose a risk of food contamination.

This study was supported by a grant from the National Science Centre, Poland, no. 2016/21/N/NZ9/01327.

FEMS7-1636
Food Microbiology - Part II

GENE PROFILING-BASED PHENOTYPING FOR IDENTIFICATION OF CELLULAR PARAMETERS THAT CONTRIBUTE TO FITNESS, ROBUSTNESS AND VIRULENCE OF ACID RESISTANT LISTERIA MONOCYTOGENES VARIANTS

J. Koomen[1], K.I. Metselaar[1], M.H. Tempelaars[1], L.M. Wijnands[2], M.H. Zwietering[1], H.M.W. den Besten[1], T. Abee[1]
[1]Wageningen University & Research, Laboratory of Food Microbiology, Wageningen, The Netherlands
[2]The National Institute for Public Health and the Environment RIVM, Centre for Zoonoses and Environmental Microbiology, Bilthoven, The Netherlands

Backgrounds

When bacterial populations are subjected to food relevant lethal stresses, such as heat and low pH, a small fraction of the population may survive. Previous studies reported isolation of stable stress resistant *Listeria monocytogenes* variants after a single exposure to acid stress. Phenotypic clustering and subsequent whole genome sequencing of the stress resistant variants revealed various mutations in *rpsU*, encoding ribosomal protein S21, in the largest phenotypic cluster.

Objectives

To elucidate features that can contribute to fitness, robustness and host interaction of stress resistant variants using a comparative gene profiling and phenotyping approach on two different *rpsU* variants.

Methods

Using a microarray we found 332 genes differentially expressed with 173 upregulated in the variants including a major contribution of Sigma-B controlled genes encoding for example the acid resistance-associated glutamate decarboxylase (GAD) system and genes involved in glycerol metabolism (*glpF*, *glpK*, *glpD*), compatible solute uptake (*OpuA*, *OpuC*) and virulence (*IntA*, *IntB*). In contrast, a strong downregulation was observed in the variants for genes involved in flagella synthesis and motility.

Conclusions

Phenotyping results matched the gene profiling data including enhanced acid resistance, higher glycerol utilisation rates, enhanced freezing resistance conceivably mediated by higher intracellular levels of compatible solutes, and better adhesion to Caco 2 cells presumably linked to higher expression of internalins. Finally, bright field and electron microscopy analysis confirmed the downregulation of flagella in the variants. Our study provides further insights in cellular parameters, that can affect robustness, fitness, and virulence of *L. monocytogenes rpsU* variants selected after exposure to food relevant stress conditions.

FEMS7-1456
Food Microbiology - Part II

HYDROGEN PEROXIDE TREATMENT OF FOOD-BORNE PATHOGENS

M. Kostrzynska[1], *J. Tang*[1], *G.Y. Mei*[1]
[1]*Agriculture and Agri-Food Canada, Guelph Research and Development Centre, Guelph, Canada*

Backgrounds

Shiga toxin-producing *Escherichia coli* (STEC) O103:H2 is one of the most common non-O157 serotypes linked to infections in humans. Hydrogen peroxide and other oxidative-based sanitizers are widely used in the food industry to control STEC and other food-borne pathogens.

Objectives

The objective of this study was to examine the response to oxidative stress in *E. coli* O103:H2 logarithmic and stationary phase planktonic cells, and in biofilms.

Methods

Transcriptional responses of *E. coli* O103:H2 stress and virulence genes induced by hydrogen peroxide treatment were examined using quantitative real-time RT PCR. Key virulence genes such as gene encoding intimin (*eae*), Shiga-toxin gene *stx1A* and flagellin gene (*fliC*) were included in this study. In addition, expression of well-known stress-associated genes such as genes involved in response to oxidative damage (*oxyR, sodA, soxR*), general stress (*uspA, rpoS*), starvation (*phoA, dps*), acid resistance (*gadW*), cold shock (*cspA, cspC, cspE*) and gene related to UV radiation stress (*mutS*) was also investigated.

Conclusions

Different gene expression patterns induced by hydrogen peroxide treatment were observed in *E. coli* O103:H2 logarithmic cells compared to biofilms and planktonic cells in stationary phase. Results derived from this study demonstrate the importance of considering biofilms and stationary phase planktonic cells when evaluating novel sanitizing methods.

FEMS7-2829
Food Microbiology - Part II

COMPARISON BETWEEN SWABBING DEVICES IN ORDER TO ANALYZE THE MICROBIAL FLORA FOUND IN SURFACES OF COMMUNITY KITCHENS BY CLASSICAL MICROBIOLOGY AND METAGENOMICS

S. Krings[1], C. Rodriguez[1], B. Taminiau[1], G. Daube[1]
[1]University of Liège - Faculty of Veterinary Medicine, Food Science Department - Food Microbiology, Liège, Belgium

Backgrounds

The work in community kitchens involves several manual steps which justifies accurate control of surfaces to prevent foodborne illnesses. Different sampling methods have been proposed to isolate bacteria from surfaces. However, recovery efficiencies of these methods are very different and not always comparable.

Objectives

The aim of this study is to evaluate different sampling methods to detect the presence of pathogenic bacteria on surfaces in contact with foods. The recovery efficiency was examined by classical microbiology an culture-independent metagenomic analysis.

Methods

To mimic surfaces which can be found in community kitchens, sterile polypropylene stainless steel surfaces and cutting boards were spiked with serial dilutions of *Escherichia coli, Salmonella enterica* and *Bacillus cereus*. They were left to dry for 1-2 hours before collection of the surviving bacteria with different moistened swabs, including commercial swabs (sponges-HS10NB2G, Sponge-Sticks-SSL100; 3M) and cotton and gauze pads. Different solutions were tested to moisten the swabs (physiological water (0,85% NaCl), Maximum Recovery Diluent and quarter-strength Ringer solution). Bacterial population analysis was performed by classical methods and metagenomics.

Conclusions

Important differences on bacterial survival were observed depending on their nature and the solution used to collect them from the surfaces. Maximum Recovery Diluent and cotton pads showed the best results in relation with bacteria recovery and required technical skills. Metagenomic analysis showed a higher detection sensibility than classical microbiology. However, preliminary results showed that both methods are necessary to improve the detection of pathogens in surfaces.

FEMS7-0364
Food Microbiology - Part II

ENTEROTOXIGENIC CLOSTRIDIUM PERFRINGENS SPORES IN U.S. RETAIL SPICES

R. Labbe[1], *C. Lee*[1]
[1]*University of Massachusetts, Food Science, Amherst, USA*

Backgrounds

Recent outbreaks of illness due to pathogens in spices has highlighted this commodity as a potential vehicle of foodborne illness. High levels of bacterial spores have long been known to be associated with spices. *Clostridium perfringens* is a leading cause of foodborne illness in the U.S. due to its ability to produce an enterotoxin. Previous surveys of its presence in spices did not assess its toxin-producing potential. The public health importance of this organism cannot be determined by its presence alone but also by the enterotoxigenicity of isolates

Objectives

To determine the levels and enterotoxigenicity of *C. perfringens* spores in U.S. retail spices.

Methods

A initial three-tube MPN procedure was used. Samples of 246 spices were diluted at 1:10, heated at 75°C, inoculated into iron milk medium and incubated at 37°C overnight. Template DNA was isolated from mid-phase cells using a boiling procedure. The presence of the enterotoxin (*cpe*) and alpha toxin (*cpa*) genes were determined using previously published primers. Spores were produced using the sporulation medium of Duncan and Strong.

Conclusions

Levels of *C. perfringens* spores ranged from 3.6 to 2400/gm from 43 of the 246 samples . Of these 27 possessed the *cpe* gene, a level much higher than the standard distribution of approximately 5% from retail, non-outbreak foods such as meat and poultry. *C. perfringens* sold in packages was isolated from 22% of samples compared to 14% purchased in bulk. Given the heat resistance of bacterial spores it is possible that spices are a potential vehicle for foodborne illness by this organism when used in food preparation.

FEMS7-2548
Food Microbiology - Part II

LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY-BASED METABOLITE PROFILING OF SALMONELLA ENTERICA BIOFILM

A. Lamas[1], *P. Regal*[1], *B. Vazquez*[1], *A. Cepeda*[1], *C.M. Franco*[1]
[1]*University of Santiago de Compostela, Analytical Chemistry- Nutrition and Bromatology, Lugo, Spain*

Backgrounds

Salmonella enterica is responsible for foodborne diseases worldwide. *Salmonella* strains persist in the food chain partially thanks to their ability to produce biofilm. Environmental conditions and surfaces affect in different ways the biofilm formation. There are few studies defining in detail the particular metabolome of these structures under different scenarios

Objectives

The aim of this study was stablishing the metabolite profile of the *Salmonella* biofilms cells growth under different conditions.

Methods

Two strains of *S.* Typhimurium and *S.* Enteritidis with great ability to produce biofilm were used. The strains were allowed to form biofilm under different environment conditions and the metabolites were measured using LC-HRMS profiling.

Conclusions

Metabolomics appears as a good tool to explore the biofilm formation pathways in *Salmonella*.

FEMS7-2552
Food Microbiology - Part II

INFLUENCE OF SURFACE, TEMPERATURE AND GROWTH MEDIUM ON THE TRANSCRIPT PROFILE OF SALMONELLA ENTERICA

A. Lamas[1], A.M. paz[1], B. Vazquez[1], A. Cepeda[1], C.M. Franco[1]
[1]University of Santiago de Compostela, Analytical Chemistry- Nutrition and Bromatology, Lugo, Spain

Backgrounds

Salmonella enterica is a major foodborne pathogen. One of the principal concerns about this microorganism in their capacity to persist in the food environment through biofilm formation. Previous studies had demonstrated the capacity of *S. enterica* to form biofilm in different surfaces as polystyrene, glass or stainless steel.

Objectives

The aim of this study is determine how different surfaces and conditions of temperature and nutrients affect to gene expression of biofilm related genes (*adr*A, *csg*D), quorum sensing genes (*lux*S and *sdi*A) and small RNA (*brc*Z, *bxy*S, *bcs*A, *rpr*A). A total of 12 strains belonging to 5 different serotypes of *S. enterica* (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Infantis, *S.* Bardo) were used in this study

Methods

The cells were able to produce biofilm in polystyrene and stainless steel surfaces and incubated at 6ºC, 28ºC and 37ºC. Two growth media were used: a high nutrient concentration media and a low nutrient concentration media. After incubation, the RNA was extracted and cDNA synthetized. Relative quantification of genes and small RNAs expression was measured using Real-Time PCR and SYBR® Green.

Conclusions

The transcript profile of biofilm cells of *Salmonella* enterica is influenced by the conditions used.

FEMS7-2022
Food Microbiology - Part II

COMPATIBILITY OF SELECTED PROBIOTIC STRAINS WITH PREBIOTIC CARBOHYDRATES AND THEIR SYNBIOTIC APPLICATION IN CHEESE

S. Langa[1], E. Van del Bulk[2], Á. Peirotén[1], P. Gaya[1], H.A. Schols[2], J.L. Arqués[1]
[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA, Tecnología de Alimentos, Madrid, Spain
[2]Wageningen University, Laboratory of Food Chemistry, Wageningen, The Netherlands

Backgrounds

To increase the beneficial effects of probiotic bacteria, prebiotics can be introduced in order to selectively stimulate the growth and/or activity of probiotics, giving rise to symbiotic products.

Objectives

The aim of this study was to test the compatibility of bacteria strains, selected based on their technological and probiotic characteristics, with different commercial prebiotic carbohydrates in order to select the optimal combination for the development of a symbiotic cheese.

Methods

The growth of three lactobacilli and one bifidobacteria strains in combination with different carbohydrates was monitored in optimized growth media and milk. Cheese was manufactured containing the selected synbiotic combination of *Lb. paracasei* INIA P272 and fructo-oligosaccharides (FOS). INIA P272 viability and physico-chemical characteristics of cheese were analysed after 28 days of ripening and compared with control cheeses.

Conclusions

Addition of FOS enhanced the growth of *Lb. paracasei* INIA P272 in optimized growth media, whereas this combination did not result in a higher number of probiotic bacteria in the synbiotic cheese. However, organic acid analysis detected higher lactic and acetic acid levels when FOS was included, suggesting an increase of *Lb. paracasei* INIA P272 metabolism upon addition of FOS to the cheese. Thus, the inclusion of FOS in the probiotic cheese could have a beneficial effect in the survival of *Lb. paracasei* INIA P272 and in host microbiota.

FEMS7-2370
Food Microbiology - Part II

HUMAN BIFIDOBACTERIUM STRAINS PERFORMANCE DURING MANUFACTURE AND RIPENING OF SEMI-HARD CHEESE

E. Mínguez[1], Á. Peirotén[1], S. Langa[1], J.L. Argués[1], P. Gaya[1], M. Nuñez[1], M. Medina[1]
[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA, Tecnología de Alimentos, Madrid, Spain

Backgrounds

Bifidobacterium strains have been commercially exploited as probiotic due to their associated health benefits and GRAS status. Bifidobacterial survival and growth require anaerobic environments, conditions found in cheese.

Objectives

Evaluation of bifidobacteria strains as adjunct cultures during manufacture and ripening of semi-hard cheeses, made with a commercial starter, and their viability after their passage through simulated gastrointestinal conditions (SGC).

Methods

B. pseudolongum INIA P2, *B. longum* INIA P678 and *B. breve* INIA P734 were selected for this study according to their good technological properties. Viability of the strains was monitored in cheese and after its exposure to SGC during the ripening period. Physico-chemical and sensory analysis of cheeses was performed after 7 and 28 d of ripening.

Conclusions

INIA P2 and INIA P734 exhibited a high survival in 28 d cheese and reductions lower than 1 log units after SGC. The addition of these strains did not affect physico-chemical quality of cheeses. In contrast, low survival of INIA P678 was observed in cheese, receiving lower scores for odour, texture and taste quality after sensory analysis. Our study suggest that *B. pseudolongum* INIA P2 and *B. breve* INIA P734 are suitable as adjunct cultures for probiotic cheese production.

FEMS7-1484
Food Microbiology - Part II

COMPLETE GENOME SEQUENCE OF GLUCONOBACTER OXYDANS GV30-1 ISOLATED FROM FERMENTED VINEGAR IN SOUTH KOREA

H.Y. Lee[1], J.H. Lee[1], H.J. Kwon[1], J.Y. Kim[1], Y.J. Goh[1], M.S. Ahn[1]
[1]Gangwondo Agricultural Research and Extension ServicesGARES, Agro-food Research Institute, Chuncheon, Republic of Korea

Backgrounds

Acetic acid bacteria (AAB) are obligate aerobes and a widely divergent group in the alpha-proteobacteria. It is unique microorganisms to oxidize alcohols such as ethanol. *Gluconobacter oxydans* GV30-1 has been used to produce traditional vinegar in many countries. We isolated *G. oxydans* GV30-1 from fermented vinegar in South Korea. The chromosome consists of 3,198,950 base pairs and contains 3,069 open reading frames. The numbers of ribosomal RNA and transfer RNA genes are 12 and 57 respectively. The GC ratio is 60.54%.

Objectives

The objectives of this study is to isolate novel acetic acid bacteria producing high acetic acid.

Methods

A total 3,198,950 bp of data corresponding to 92,319 reads with Pacbio 20kb. For assembly, we used CLC Genomics workbench 6.5.1.

Conclusions

The genome analysis of *A. G. oxydans* GV30-1 provided detail insights into understanding metabolic fermentation of *G. oxydans* GV30-1. We will study more genetic information to manufacture high-quality of vinegar.

FEMS7-1494
Food Microbiology - Part II

CHARACTERIZATION AND PURIFICATION OF THE NOVEL BACTERIOCIN PRODUCED BY BACILLUS SP. AMP-18

J.Y. Lee[1], *D.O. Kang*[1]
[1]*Changwon National University, Department of Bio Health Science, Changwon-si, Republic of Korea*

Backgrounds

Bacteriocins are ribosomally synthesized by diverse bacteria exhibit antibacterial activity against bacteria closely related to producer strains. Korean traditional foods are good source for screening bacteriocin-producing bacterial strains.

Objectives

This study was performed to obtain a novel bacteriocin from bacterial strains isolated from fermented foods. We characterized and purified the bacteriocin.

Methods

We collected various fermented foods and isolated bacteria, followed by cultivation at 37°C, 24-h. The antibacterial activity of culture supernatant was confirmed by agar well diffusion method. The physicochemical properties such as heat, pH and solvent resistance were studied. The bacteriocin was purified via three steps. Molecular weight and N-terminal amino sequences were determined.

Conclusions

Maximum inhibitory activity were achieved at an initial pH of 7.5 and at 37°C for 24-h growth. The antibacterial activity was retained at the pH range from 2.0 to 8.0 but decreased more than at 8.0. The bacteriocin activity was stable at up to 80°C for 40 min but 80% of activity was lost at 100°C for 10 min. The bacteriocin was highly resistant to solvents such as acetonitrile, methanol, isopropanol at up to 100% concentration but sensitive to 100% butanol. Its molecular weight was determined to be 3.4 kDa by mass spectrometry. The 15-N-terminal amino acid sequences of the purified bacteriocin were determined to be L-P-I-P-A-D-L-V-D-G-P-X-G-P-R. Amino acid sequence alignment analysis showed this bacteriocin is possibly novel one. The bacteriocin exhibited its antibacterial activity against through bacteriostatic action. The bacteriocin inhibited growth of *Listeria monocytogenes* KCCM 40307 in beef stored at 4°C for 15 days.

FEMS7-1619
Food Microbiology - Part II

SYNERGISTIC EFFECT OF DNASE I PRETREATMENT COMBINED WITH SODIUM HYPOCHLORITE SANITIZER TREATMENT IN INACTIVATION OF CAMPYLOBACTER BIOFILMS

J.Y. Lee[1], J.S. Kim[2]
[1]Korea Food Research Institute, Food Analysis Center, 1201 Anyangpangyo-ro- Bundang-, Republic of Korea
[2]Korea Food Research Institute, Research Group of Food Safety, 1201 Anyangpangyo-ro- Bundang-gu- Seong-nam si, Republic of Korea

Backgrounds

Campylobacter jejuni is a foodborne pathogenic bacterium associated with poultry. It can form biofilms on food contact surfaces, which can pose a raised threat because biofilms are likely to be highly resistant to environmental stresses including sanitizers. Therefore, an effective control method needs to be developed for *Campylobacter* biofilms.

Objectives

The objective of this study was to evaluate synergistic effect of DNase I and sodium hypochlorite combinations in inactivation of *campylobacter jejuni* biofilms.

Methods

The biofilms of two *Campylobacter jejuni* strains, NCTC 11168 reference strain and a strain, Y23-5 from chickens were evaluated for sensitivities to DNase Ⅰ and sodium hypochlorite treatments. Biofilms grown in 96-well plates were pre-treated with DNase I with subsequent sodium hypochlorite treatment (10 or 20 ppm). The detached cells after vigorous shaking were serially 10-fold diluted up to 10^{-4}, then 100 ul aliquots were spread on blood agar plates, and the colonies were enumerated after incubation.

Conclusions

In strain 11168, neither DNase I or sodium hypochlorite treatment was effective in inactivation of biofilms. However, sodium hypochlorite treatment (10 or 20 ppm) after DNase I pre-treatment effectively inactivated biofilms by 2.7 log CFU and more than 4.3 log CFU at 10 ppm and 20 ppm, respectively ($P<0.05$). Similarly, in strain Y23-5, sodium hypochlorite treatment (10 ppm) after DNase I pre-treatment effectively inactivated the biofilms by more than 3.5 log CFU ($P<0.05$), while either method was not effective when used alone. This study clearly shows that DNase I combined with Sodium Hypochlorite has a synergistic effect in inactivation of *Campylobacter* biofilms.

FEMS7-0819
Food Microbiology - Part II

PHYLOGENETICAL AND PAN GENOME ANALYSES OF FOOD-BORNE PATHOGENS, BACILLUS CEREUS ISOLATED FROM SOUTH KOREA

Y.T. Kim[1,2], H. Kim[2,3], S. Ryu[2,3], S.H. Choi[2,3], J.H. Lee[1,2]
[1]Kyung Hee University, Food Science and Biotechnology, Yongin, Republic of Korea
[2]Seoul National University, Food-borne Pathogen Omics Research Center-FORC, Seoul, Republic of Korea
[3]Seoul National University, Food and Animal Biotechnology, Seoul, Republic of Korea

Backgrounds

Pan genome analysis is useful technique to compare whole genome sequences. It offers core proteins and strain specific proteins to users. Recently, it used to compare complete genomes of *Bacillus cereus* strains isolated from food samples causing food-borne outbreaks in South Korea with other foreign *B. cereus* genomes.

Objectives

Nine genomes of *B. cereus* were obtained from FORC genome database and comparatively analyzed to reveal distinct features between South Korean *B. cereus* and other foreign *B. cereus* strains in phylogenetic and Pan-genome level.

Methods

Nine genomes of South Korean *B. cereus* strains were completely sequenced and assembled using PacBio system. The open reading frames (ORFs) were predicted by merging with GeneMarks and Glimmer, and their functions were annotated using BlastP and Interproscan. Other *B. cereus* genome information was obtained from GenBank database. Comparative genome analysis was performed using JSpecies program for average nucleotide identity (ANI) analysis and Roary program for Pan-Genome analysis.

Conclusions

The phylogenetical tree using ANI method showed that *B. cereus* has two phylogenetic tree groups, Group I and II. Interestingly, all South Korean *B. cereus* strains belong to the Group II . Most of *B. cereus* strains in the Group II are clinical isolates with high pathogenicity, suggesting that all South Korean *B. cereus* are pathogenic indeed. In addition, Group II consists of four subgroups in the phylogenetic tree. To clarify and determine the distinct features of strains in the subgroups, their pan-genome analysis was performed. This analysis revealed that each subgroup strain has different specific genes. Based on these comparative results, Group- or subgroup-specific primer sets targeting specific genes can be designed. Using this specific PCR primer set, Group I vs. II as well as each subgroup in the Group II will be able to be distinguished using specific PCR to identify the isolated *B. cereus* strain quickly.

FEMS7-1348
Food Microbiology - Part II

SELECTION, VERIFICATION, PARTIAL OPTIMIZATION OF IN VITRO EFFICACY OF ANTI-AFB1 ACTIVITY OF CHRYSEOBACTERIUM SP. C4A USING EXTRACELLULAR FRACTION AND ISOLATION OF ACTIVE PROTEIN

A. Limaye[1], *J.R. Liu*[2,3,4]
[1]*Institute of Biotechnology, National Taiwan University, Taipei, Taiwan R.O.C.*
[2]*Institute of Biotechnology, National Taiwan Iniversity, Taipei, Taiwan R.O.C.*
[3]*Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan R.O.C.*
[4]*Department of Animal Science and Technology, National Taiwan University, Taipei, Taiwan R.O.C.*

Backgrounds

Aflatoxins, the difuranocoumarin derivatives; are the hazardous mycotoxins secreted mainly by the filamentous fungi *Aspergillus flavus* as secondary metabolites. Among different aflatoxins, aflatoxin B1 (AFB1) is identified as the most toxic hepatocarcinogen.

Objectives

Our objectives are 1) to screen and select best AFB1 degrading bacteria, 2) to identify degradation metabolites, and 3) to identify anti-AFB1 active compound and responsible gene.

Methods

Using coumarin minimal selection medium we screened for AFB1 degraders from chicken ileo-cecal digesta sample. Additionally we also compared coumarin degrading potential for keratinolytic soil bacterium *Chryseobacterium* sp. c4a. The cell free anti-AFB1 activity was validated by MALDI/TOF and RP-HPLC as qualitative and quantitative methods respectively. Anti-AFB1 activity of c4a strain was partially optimized for optimum reaction temperature, pH, theromstability and essential metal co-factors. We used a UPLC/LTQ Orbitrap LC/MS to analyze chemical profile of cell free supernatant in presence of AFB1. Upon protein concentration of active cell free fraction using tangential flow filtration, we performed the FPLC size exclusion chromatography followed by AFB1 degradation for all collected fractions.

Conclusions

Strain c4a demonstrated exclusively extracellular, constitutive anti-AFB1 degradation ability. It showed upto 85% AFB1 degradation for concentrations exceeding to 2 ppm when originally grown in plain nutrient broth containing gelatin. HPLC profile showed a cleavage of original AFB1 peak. LC/MS/MS analysis showed bixin as a major metabolite present in active fraction which is a natural apocarotenoid. Currently, we are sequencing the whole genome of c4a strain using NGS along with protein purification and anti-AFB1 gene identification studies to establish its anti-AFB1 role.

FEMS7-0192
Food Microbiology - Part II

ENDOPHYTIC FUNGUS FUSARIUM GRAMINEARUM AS A BIOSYNTHESIS FACTORY OF SILVER NANOPARTICLES WITH BROAD ANTIMICROBIAL ACTIVITY

X. Liu[1], H. Peng[2], Y. Hu[1], Y. Liu[2], J. Zhou[1]
[1]Jiangsu Academy of Agricultural Sciences, Institution of Argo-product Processing, Nanjing, China
[2]Shanghai Ocean University, College of Food Science and Technology, Nanjing, China

Backgrounds

Synthesis of nanoparticles, especially silver nanoparticles (AgNPs) with the antimicrobial activity, is an emerging field. But conventional physical and chemical methods proved to be toxic due to the aid of reducing agents or under extreme temperature and pressure conditions. Green synthesis using microorganisms could be an attractive advancement.

Objectives

This study aims to isolate and identify an endophytic fungus from *Dendrobium candidum*, and to evaluate its biosynthesis capacity of antimicrobial silver nanoparticles(AgNPs).

Methods

The fungus, isolated from the stem of *D.candidum* after surface sterilization, was identified based on internal transcribed spacer (ITS) sequence and morphological traits. The filtrate solution of the fungal biomass in distilled water was subjected for extracellular biosynthesis of AgNPs. The AgNPs were characterized by UV-visible spectroscopy and transmission electron microscopy (TEM), and were tested for antimicrobial activity by the agar well diffusion method against several food-borne pathogens including bacteria and fungi.

Conclusions

Endophytic fungus DO-1 was identified as *Fusarium graminearum*. UV-visible spectroscopy showed AgNPs had the surface plasmon resonance at 440~450 nm. Darkness and suitable high temperature (80 ºC) were helpful for the biosynthesis of AgNPs and the increase of antimicrobial activity. As the increase of pH, the absorbance at 440~450 nm increased, but the highest antimicrobial activity was observed at pH 7~8. TEM revealed AgNPs to be spherical with the diameter of 2~50 nm. The antimicrobial activity of AgNPs against the tested pathogens showed the inhibition zone ranging from 11 to 18 mm. The biosynthesis of AgNPs using endophytic *F.graminearum* in a facile way is simple and eco-friendly.

FEMS7-0208
Food Microbiology - Part II

GALACTOMYCES GEOTRICHUM ISOLATED FROM KEFIR GRAINS: ADHESION INTERACTION WITH OTHER BACTERIA

X. Liu[1], H. Peng[2], Y. Hu[1], J. Zhou[1]
[1]Jiangsu Academy of Agricultural Sciences, Institution of Argo-product Processing, Nanjing, China
[2]Shanghai Ocean University, College of Food Science and Technology, Shanghai, China

Backgrounds

Kefir grains possess a complex and stable microbiota association. Current studies on the microbial population and symbiotic interactions laid special emphasis on the role of lactic acid bacteria. There's no detailed report about the role of fungi.

Objectives

This study aims to investigate the adhesion properties of a yeast-like fungus obtained from kefir grains, and to evaluate its role on the microflora formation of kefir grains.

Methods

The isolate KG-1 was identified morphologically and phylogenetically. The adhesion ability of the fungus with other bacteria was observed using optical microscope and scanning electron microscope observation. Different pretreatments, including heat, sodium hydroxide (NaOH), acetic acid, and lithium chloride (LiCl), were used to evaluate the adhesion activity of the nonviable or partially damaged cells. Auto-aggregation, co-aggregation abilities and cell surface hydrophobicity were determined spectrophotometrically.

Conclusions

The fungus KG-1, identified as *Galactomyces geotrichum*, had great adhesive activity with various bacteria. Exposure to LiCl aroused the marked loss of attached bacteria, while acetic acid treatment led to a thicker adhesion. Heat and NaOH treatment had non-significant effect on the interaction. A strong affinity to xylene (83.49%) and chloroform (87.5%) as well as a low affinity to ethyl acetate (42.22%) indicated the hydrophobic and basic phenotype of *G. geotrichum* KG-1, with strong electron donor property, contributing to the extremely strong auto-aggregation ability (97.48%). Different treatments changed the surface properties greatly. The unique adhesion and aggregation properties of *G.geotrichum* contribute greatly to the biofilm and grain formation in kefir.

FEMS7-0955
Food Microbiology - Part II

LYOPHILIZATION: EFFECT ON HUMAN MILK BACTERICIDAL PROPERTIES AND EFFECT ON MICROBIAL OCCURRENCE

M. Lozano Fuentes[1], L.M. Carmen[1], M. Gormaz[2], J. Salcedo[3], S.C. Dolores[1]
[1]UNIVERSIDAD CEU CARDENAL HERRERA,
ANIMAL PRODUCTION- ANIMAL AND PUBLIC HEALTH- FOOD TECHNOLOGY AND SCIENCE, Alfara del Patriarca, Spain
[2]Hospital Universitario La Fe de Valencia, Banco de Leche Humana, Valencia, Spain
[3]UC Davis, UC Davis, Davis, USA

Backgrounds

Lyophilization is highly valued as a storage technique for complex products that require quality guarantees. Thus, it may be a good alternative to storing Human Milk (HM) in Human Milk Banks (HMB) because this technique, can offer maximum preservation of the properties of the original product, such as bactericidal properties.

Objectives

Study of the effects of lyophilization in HM as a method of storage in HMBs on mesophilic aerobic microorganisms occurrence and HM bactericidal properties.

Methods

Lyophilization

A total of 125 HM samples from 65 healthy donors in the HMB of La Fe Hospital (Valencia, Spain) were analyzed. Each sample collected, was thawed and divided into 2 aliquots of 10ml each. One of this was used to lyophilization (Telstar Cryodos, Mannheim) and the other aliquot was used as a control, freezing at -20°C.

Microbial occurrence

Mesophilic aerobic counts were determined in each aliquot using plate count agar and incubating at 31°C for 72 hours. The results were expressed as colony-forming units per milliliter (CFU/ml).

Determination of Bactericidal Capacity

The bactericidal effect against *Escherichia coli* was assessed using the method described by Silvestre et al (2006) [1].

[1] Silvestre D., Lopez M.C., March L., Plaza A. and Martinez-Costa C. 2006. Bactericidal activity of human milk: stability during storage. *British Journal of Biomedical Science*, 63(2):59-62

Conclusions

Lyophilization significantly lowered the counts of mesophilic aerobic microorganisms compared with freezing at -20°C (*average*= $29 \cdot 10^3$ CFU/mL in frozen samples at -20ºC and *average* = $15 \cdot 10^3$ CFU/mL in lyophilized samples). In the other hand, there are not significant differences of bactericidal capacity attributable to the storage method used.

FEMS7-0264
Food Microbiology - Part II

ISOLATION AND CHARACTERIZATION OF A BACTERIOPHAGE AGAINST SALMONELLA ENTERICA SEROVAR MONTEVIDEO

Z. Lu[1], S. Kim[1], F. Breidt[2], J. Niedermeyer[3], S. Kathariou[3]
[1]Kennesaw State University, Molecular & Cellular Biology, Kennesaw, USA
[2]NC State University and USDA/ARS, Food- Bioprocessing- and Nutrition Sciences, Raleigh, USA
[3]North Carolina State University, Food- Bioprocessing- and Nutrition Sciences, Raleigh, USA

Backgrounds

Salmonella enterica serovar Montevideo is globally distributed. It is among the most common serovars isolated from humans and animals. Numerous foodborne outbreaks in different countries have been linked to *S*. Montevideo. Effective control of *S*. Montevideo is important to public health. Using bacteriophages to control foodborne bacterial pathogens is a promising novel biocontrol method.

Objectives

The objectives of this study were to isolate and to characterize a bacteriophage infecting *Salmonella* serovar Montevideo.

Methods

The phage (named ΦMont) was isolated from turkey feces and characterized based on its morphology, host range, one-step growth kinetics, and structural protein profile.

Conclusions

Phage ΦMont formed very large clear plaques on its host lawn. Transmission electron microscopy revealed that the phage belongs to the *Podoviridae* family. Host range analysis showed that the phage was serovar-specific and only infected *S*. Montevideo, *S*. Infantis, and *S*. Braenderup among 20 tested *Salmonella* serovars. The phage infection led to very rapid cell lysis. One-step growth kinetics study showed that the latent period was 20 min (including 10 min for adsorption) and the average burst size was 70,000 phage particles per infected cell. SDS-PAGE profile of the phage revealed six structural proteins ranging from 20 KDa to 80 KDa. The large burst size, high lytic activity, and narrow host range suggested that phage ΦMont has a high potential for use as a biocontrol agent specifically against *Salmonella enterica* serovar Montevideo in food systems.

FEMS7-0932
Food Microbiology - Part II

THE MICROBIOTA OF LUTEFISK, A TRADITIONAL NORWEGIAN COD DISH WITH A HIGH PH

B.T. Lunestad[1], D.H. Grevskott[1], I.S. Roiha[1], C.S. Svanevik[1]
[1]National Institute of Nutrition and Seafood Research, Section for contaminants and biohazards, Bergen, Norway

Backgrounds

Lutefisk is a fish preparation with long traditions in Norway, especially during Christmas time. It is produced from dried cod soaked in water for six to seven days, then transferred to lye for two to four days and put in water to dilute the lye. Traditionally the lye was made by boiling 1 L of wood ash in 2.5 L water. As the muscle are partially digested, *lutefisk* have a gel like appearance and characteristic smell, both appreciated by dedicated connoisseurs. A *lutefisk* meal is commonly accompanied by boiled potatoes, Swedish stew, pees, fried bacon and mustard.

Objectives

The objective of this study was to examine the microbiota of *lutefisk*.

Methods

Four commercially available *lutefisk* were purchased during December 2016, and examined for plate count (aerobic and anaerobic), indicator organisms for faecal contamination, staphylococci and specific pathogens. Colonies with unique appearance on different agar plates were grown into pure cultures and identified by 16S Sanger sequencing and BLAST alignment with <97% hit.

Conclusions

Despite a pH above 10.5 in all samples, the *lutefisk* had aerobic and anaerobic counts of up to $1.2x10^6$ and $3.0x10^4$, respectively. The fish did not harbour indicator organisms of faecal contamination or pathogens in the genera *Listeria*, *Salmonella* or *Vibrio*. The pure culture isolates were identified as *Carnobacterium maltaromaticum*, *Pseudomonas fragi*, *Staphylococcus warneri, S. hominis, Aeromonas salmonicida*, *Kurthia zopfii*, *Microbacterium oxydans*, *Exiguobacterium oxidotolerans*, *Alishewanella* sp. and *Bacillus licheniformis*.

FEMS7-2836
Food Microbiology - Part II

LACTIC ACID BACTERIA WITH POTENTIAL FOR OCHRATOXIN A DEGRADATION IN VITRO

C. Luz Mínguez[1], J. Ferrer Roig[1], F. Saladino[1], F.B. Luciano[2], J. Mañes[1], G. Meca[1]
[1]Universidad de Valencia, Medicina Preventiva, Burjassot, Spain
[2]Pontificia Universidade Católica do Paraná,
Departamento de ciência animal- Escola de Ciências da Vida, Curitiba, Brazil

Backgrounds

Ochratoxin A (OTA) is a mycotoxin produced by the metabolism of fungus belonging to the genus *Aspergillus* and *Penicillium*. It is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (group 2B) and it has been proven to be nephrotoxic and hepatotoxic, amongst other toxic properties. Human exposure to OTA occurs mostly by dietary intake, since it's a common contaminant in many foodstuffs such as cereal and coffee grains, grapes, etc. In the same way, the possibility that farming animals may result intoxicated by exposure to contaminated agricultural products and fodder represents a risk for public health.

Objectives

In the present study, the capacity of different cultures of lactic acid bacteria (LAB) to degrade OTA present in Man Rogosa Sharpe (MRS) liquid medium, specific for LAB growth, was evaluated.

Methods

For that purpose, 17 different bacteria strains, most of them provided by the *Colección Española de Cultivos Tipo* (CECT), were grown in MRS broth at both pH 3.5 and 6.5, contaminated with 1mg/L of OTA. The tubes were kept at 37°C for 48h. After that, the samples were centrifuged and the remaining OTA was quantified, both in the cell-free supernatant (CFS) and in the cellular precipitate.

Conclusions

The results obtained show that the OTA concentration in the mediums suffered a total reduction up to 98% when the pH was 6.5 and 97% at pH 3.5. The reduction is mostly due to molecular hydrolysis of the OTA, while the cell-adsorption mechanism seems to have little influence.

FEMS7-0440
Food Microbiology - Part II

CHARACTERIZATION OF A SS-GLUCOSIDASE ISOLATED FROM AN ALPEORUJO STRAIN OF CANDIDA ADRIATICA

J. Lilao[1,2], S. Callejón[1], J.J. Mateo[1], S. Maicas[1]
[1]Universitat de València, Microbiologia i Ecologia, Burjassot, Spain
[2]University of Bath, Biology and Biochemistry, Bath, United Kingdom

Backgrounds

Olives have a bitter compound namely oleuropein, which should be removed during the table olives and the olive oil production process. Industrial debittering procedures comprise treatment of the olives with a sodium hydroxide solution to hydrolyze the oleuropein. A substitute strategy is based on biological debittering when microbial ß-glucosidases participate in hydrolysis of oleuropein.

Objectives

The aim of the present work was to characterize the ß-glucosidase from a strain of *Candida adriatica* isolated from an olive oil mill. The isolation, purification and characterization of the enzyme could generate valuable information for the commercial use of this enzyme.

Methods

A total of 216 yeast strains were isolated from alpeorujo samples in an olive mill located in Villamalea (Eastern Spain) during the 2012 olive oil production campaign. The isolates were analysed by RFLP analysis of the 5.8S-ITS rDNA region. For sequence analysis of the D1/D2 domains of the 26S rDNA gene, PCR amplification was performed. Enzyme purification and basic characterization (pH, T, kinetic constants, metal ion effects) of a selected strain was carried out.

Conclusions

The isolation, purification and characterization of ß-glucosidase derived from non-*Saccharomyces* yeasts are of biotechnological interest because this enzyme is involved in the degradation of oleuropein for the debittering of table olives and olive oil. This study shows the potential of the ß-glucosidase produced by *C. adriatica* CECT13142 in this process.

Grants: This work was supported by the project “Identification and biotechnological characterization of yeasts isolated from agrifood residues of the Valencian Community”, grant AICO-2016-079.

FEMS7-0486
Food Microbiology - Part II

EXOCELLULAR PROTEASES PRODUCED BY ENOLOGICAL HANSENIASPORA ISOLATES CAN BE USED FOR BIOTECHNOLOGICAL INDUSTRIES

C. Thießen[1], S. Maicas[1], J.J. Mateo[1]
[1]Universitat de València, Microbiologia i Ecologia, Burjassot, Spain

Backgrounds

Hanseniaspora are yeasts mainly found in soil, on fruits and trees and in spoiled foods and beverages. They are characterized by apiculate cells with vegetative reproduction by bipolar budding in basipetal succession. The six validly described species in the genus are physiologically very similar; they ferment glucose, assimilate few carbon compounds (arbutin, cellobiose, glucose, glucono-δ-lactone and salicin), and require inositol for growth. Most of these species have been previously reported in wine and grape juice. From a biotechnological point of view, although these yeasts can produce spoilage of fruits, they also possess many interesting technological properties. In this way, esterases, glycosidases, lipases, proteases, catalase of relative yeast species in different ecosystems have been previously described in our laboratory.

Objectives

The aim of this work was to characterize, from a biotechnological point of view, exocellular proteases produced by *Hanseniaspora* yeasts isolates from grape juice in order to evaluate the suitability of these proteins to be used in industrial processes.

Methods

Molecular identification was carried out by rDNA sequencing. Exocellular protease production was determined by spreading yeast colonies onto YPD agar plates containing 20 g/L casein. Effect of sugars, ethanol, temperature, pH, metal ions and other compounds on protease activity were obtained after standard laboratory procedures.

Conclusions

This is, to our knowledge, the first work showing the induction process to induce proteolytic activity in *Hanseniaspora* isolates. In this study some isolates have been characterized, and they are now available to be used in biotechnological processes.

This work was supported by the project “Identification and biotechnological characterization of yeasts isolated from agrifood residues of the Valencian Community”, grant AICO-2016-079.

FEMS7-2005
Food Microbiology - Part II

DIVERSITY DYNAMICS OF THE FERMENTATION OF IDLI BATTER

M. Mandhania[1], *M. Suryavanshi*[2], *M. Patole*[1]
[1]*National Centre For Cell Science, Pathogenesis And Cellular Response, Pune, India*
[2]*National Centre For Cell Science, Microbial Ecology, Pune, India*

Backgrounds

Idli, a fermented food of India, prepared from rice (*Oryza sativa*) and black gram (*Phaseolus mungo*), consists of a consortium of micro-organisms, which initiate a natural fermentation, leading to acidification and leavening of the *Idli* batter. The steamed *Idli* cakes prepared from the fermented batter are popular for their nutritional content, unique taste and appetizing aroma. However, a detailed analysis of the microbial community structure responsible for fermentation of *Idli* batter has received little attention.

Objectives

To determine the microbial population enabling the fermentation process and also study the microbial succession patterns involved over the course of 24h fermentation.

Methods

In order to study the microbial diversity responsible for *Idli* batter fermentation, 16S rRNA V3 region amplicon sequencing was performed. Polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) was used to analyse the microbial succession pattern based on 16S rRNA partial sequences of the microflora. The abundant genera were also confirmed by q-PCR.

Conclusions

Analysis of the 16S rRNA gene sequencing results by QIIME pipeline revealed that the fermentation process was typically dominated by the hetero-fermentative bacterial genus *Weissella*. Using PICRUSt for microbial functional composition analysis, an enrichment of metabolic functional features including amino acid and carbohydrate metabolism was observed, implying that vigorous microbial metabolism occurred during the fermentation process. Comprehensively, our results illustrate the dynamic nature of *Idli* batter fermentation and microbial succession pattern therein and can be applied to optimize the fermentation processes, flavours and health-related attributes of this and other fermented foods.

FEMS7-3008
Food Microbiology - Part II

GENETIC CHARACTERIZATION OF LISTERIA MONOCYTOGENES ISOLATES FROM FOOD AND FOOD ENVIRONMENT

B. Manso[1], B. Stessl[2], M. Wagner[2], J. Rovira[1], B. Melero[1], D. Rodríguez-Lázaro[1]
[1]University of Burgos, Biotechnology and Food Science, Burgos, Spain
[2]University of Veterinary Medicine, Institue for Milk Hygiene- Milk Technology and Food Science, Vienna, Austria

Backgrounds

Listeria monocytogenes is a major foodborne pathogen that can survive frequently in food processing environments.

Objectives

The aim of this study was to characterize 15 strains of *L. monocytogenes* of different sequence types (ST) and sources.

Methods

Thirteen isolates were from a poultry slaughterhouse -ST1 (15.38 %), ST5 (7.69 %), ST8 (7.69 %), ST9 (15.38 %), ST87 (7.69 %), ST121 (15.38 %), ST199 (7.69 %) and ST388 (7.69 %) - and 2 were seafood isolates -ST2 and ST321-. The characterization consisted of the identification by PCR of different genetic markers related with the environmental stress adaptation and virulence; the presence of Stress Survival Islet (SSI-1), listeriolysin S (*lls*), Internalin A truncation (*inl*A) and tolerance to benzalkonium chloride due to Transposon 6188 and *bcr*ABC cassette gene.

Conclusions

ST1, ST2, ST5, ST87 and ST388 were the most virulent isolates, as they were positive to Listeriolysin S and possess the complete Internalin A. Only the isolates belonging to ST9 and ST8 had the 10 Kb fragment of SSI-1, and only a fragment of 2Kb was detected in the ST121 isolate, but they showed a truncated Internalin A. Only the ST121 isolates had Tn 6188, with the exception of one isolate, and the ST2 and ST321 isolates were positive to *bcr*ABC cassette being usually, environmental.

Results showed that *L. monocytogenes* isolated from food processing environment not only have genetic markers that could allow the pathogen to survive better in the environment but also have virulence markers increasing the risk of listeriosis in the consumers.

FEMS7-3009
Food Microbiology - Part II

EFFECT OF SANITIZERS AND DISINFECTANTS USED IN FOOD INDUSTRY ON LISTERIA MONOCYTOGENES GROWTH

B. Manso[1], I. Jaime[1], A.M. Diez[1], J. Rovira[1], D. Rodríguez-Lázaro[1], B. Melero[1]
[1]University of Burgos, Biotechnology and Food Science, Burgos, Spain

Backgrounds

Listeria monocytogenes is a foodborne pathogen able to adapt to food processing environmental conditions such as sanitizer agents due to some genetic markers.

Objectives

The aim of this study is to know the behavior of *L. monocytogenes* isolates in the presence of an acid sanitizer and a disinfectant based on ammonium quaternary compounds (QAC).

Methods

Seven strains of *L. monocytogenes* were isolated from food products and environmental surfaces from 3 different food industries: a poultry slaughterhouse, a cheese factory and a seafood company**.** The behavior was analyzed in the presence of different concentrations of the detergent (2 %, 1 %, 0.25 %, 0.1 %, 0.05 % and 0.0025 %) and the disinfectant (0.0125 %, 0.00625 % and 0.003125 %). Isolates were inoculated at 10^7 cfu/mL and the growth was measured at OD_{600} during 24 hours at 37 °C.

Conclusions

All the strains were sensitive to the highest detergent concentration but they grew similar to the control in the presence of the remaining concentrations**.** Only 2 isolates were resistant to 0.0125 % of disinfectant although all the isolates were tolerant to the lowest concentration**.**

L. monocytogenes could tolerate benzalkonium chloride (QAC derivated) and antibiotics because of its ability to exchange genes with other microorganisms. These molecular mechanisms are activated during lag phase, when *L. monocytogenes* needs to growth on the stressful environment.

Results showed the importance of applying good hygiene and disinfection protocols to avoid the contact of *L. monocytogenes* with sublethal concentrations that lead to an increasing resistance of the pathogen in food industries.

FEMS7-0562
Food Microbiology - Part II

HIGH-THROUGHPUT SEQUENCING ANALYSIS OF THE MICROBIOTA INVOLVED IN SPANISH DRY FERMENTED PORK SAUSAGE ("CHORIZO") RIPENING

N.M. Quijada[1], F. De Filippis[2], J.J. Sanz[3], D. Rodríguez-Lázaro[4], D. Ercolini[2], M. Hernández[1]
[1]Instituto Tecnológico Agrario de Castilla y León ITACyL,
Laboratory of Molecular Biology and Microbiology, Valladolid, Spain
[2]University of Naples Federico II, Department of Agricultural Sciences, Portici, Italy
[3]Instituto de Ciencia y Tecnología de los Alimentos ICTAL, Microbiology, León, Spain
[4]Universidad de Burgos, Department of Biotechnology and Food Science, Burgos, Spain

Backgrounds

Fermentation is one of the oldest technologies used to preserve food for long periods. It involves different microorganisms, such as lactic acid bacteria, that are responsible of improving safety and organoleptic properties. "*Chorizo"* is the most popular Spanish dry fermented pork sausage. High-Throughput Sequencing (HTS) targeting 16S rRNA gene allows deep investigation of the microbial ecology of different environments saving time and money, respect to classic microbiological methods. New approaches such as oligotyping allow sub-genus classification and avoid the bias of clustering-based methods.

Objectives

This study aims to elucidate the microbiota involved in the ripening process of *"chorizo"* using HTS technologies.

Methods

"Chorizo" samples were taken from 6 factories at different time points along its production. Total DNA was extracted, 16S rRNA gene amplified and sequenced using Illumina platform. Quality-filtered sequences were analyzed by using QIIME 1.9.0 and sub-genus level analysis of *Lactobacillus*-assigned sequences was performed by using oligotyping package v.2.1.

Conclusions

"Chorizo's*"* microbial composition changes along the ripening process. The first steps (when the meat is minced and marinated) are characterized by a high bacterial diversity where several genera (*Bacillus, Staphylococcus* and the air storage-related spoilers *Pseudomonas* and *Brochotrhyx*) can be observed in high abundance. Sausage production (and thus ripening starts) makes a shift in the microbial composition, where *Lactobacillus* develops and becomes the most abundant genus at the end of the process. Sub-genus level investigation revealed that the most abundant oligotype correspond to *Lactobacillus sakei*, one of the most frequently bacteria isolated from fermented meat products.
(Aknowledgments: ESF and INIA RTA2014-00024-C04-01)

FEMS7-2291
Food Microbiology - Part II

PROPIDIUM MONOAZIDE (PMA) COUPLED WITH QPCR ENABLES THE DETECTION OF INFECTIOUS VIRUSES IN FOOD

N.M. Quijada[1], G. Fongaro[2], C.R.M. Barardi[2], M. Hernández[1], D. Rodríguez-Lázaro[3]
[1]Instituto Tecnológico Agrario de Castilla y León ITACyL,
Laboratory of Molecular Biology and Microbiology, Valladolid, Spain
[2]Universidade Federal de Santa Catarina,
Departamento de Microbiologia- Inmunologia e Parasitologia, Florianópolis, Brazil
[3]Universidad de Burgos, Department of Biotechnology and Food Science, Burgos, Spain

Backgrounds

PCR and qPCR appeared as promising alternatives for food safety monitoring, but they are not able to discern between infectious/non-infectious viruses. Propidium monoazide (PMA) is a nucleic acid intercalating dye that only crosses damaged lipid membrane barriers and binds covalently to DNA/RNA after exposure of strong visible light, interfering DNA amplification. Entire-lipid membrane genomes are not affected by PMA and DNA can be amplified with PCR.

Objectives

This study assesses the use of PMA with qPCR/RT-qPCR to distinguish between infectious and non-infectious viruses in two food matrices: clam and Spanish dry fermented pork sausage ("*chorizo*").

Methods

Clam and *"chorizo*" samples were artificially contaminated with decreasing amounts of infectious and thermally inactivated adenovirus-2 (HAdV-2) and mengovirus (vMC_0) (representatives of DNA and RNA viruses, respectively). Samples were processed and mixed or not with PMA. Photo-activation was performed in treated samples. Nucleic acids were extracted and qPCR/RT-qPCR was performed. Infectious viruses were quantified by plaque assay (PFUs) and results were compared to those obtained by qPCR/RT-qPCR.

Conclusions

No differences were shown between infectious-contaminated samples regarding its treatment with PMA. Significant reductions in GCs were observed in thermally inactivated-contaminated samples when were treated with PMA prior qRT/RT-qPCR. IT represented a mean PCR derived signal reduction of 99.96 and 99.92% for HAdV-2 and 99.70 and 99.93% for vMC_0, in clam and *"chorizo"*, respectively. Linear regression (R^2 from 0.92 to 0.99) demonstrated correlation between PFUs-GCs. PMA coupled with qPCR/RT-qPCR represent a promising alternative for virus investigation in food safety, especially for non-cultivable viruses.

FEMS7-2086
Food Microbiology - Part II

ANTIMICROBIAL SUSCEPTIBILITY AND PATHOGENICITY OF C. COLI STRAINS ISOLATED FROM HUMANS AND CHICKEN FOOD CHAIN

A.J. Martinez-Rodriguez[1], *D. Perez-Boto*[1], *A. Garcia-Bravo*[1]
[1]*Institute of Food Science Research CIAL- CSIC-UAM, Microbiology and Biotechnology, Madrid, Spain*

Backgrounds

Campylobacter is the leading cause of bacterial food-borne gastroenteritis worldwide and the species *C. jejuni* and *C. coli* cause more than 95% of the infections attributed to this genus. In the European Union, campylobacteriosis was the most commonly reported bacterial zoonosis, with 236,851 confirmed human cases in 2014. Although *C. jejuni* is the most diagnosed species, in the last years C*. coli* has become increasingly important, mainly due to the high antibiotic resistant levels.

Objectives

To study the incidence and virulence of *C. coli* and its relationship with the human infection in 201 *Campylobacter* strains isolated from the chicken food chain and humans patients.

Methods

The response to 14 different antibiotics was analyzed following the Kirby-Bauer. The results obtained showed that *C. coli* had the highest levels of drug multiresistance (MRD) with an 84.6%. They also presented a 20.5% of resistance to erythromycin (Ery-R) among human isolates, the first therapeutic option in campylobacteriosis. The presence of three relevant genes associated to *Campylobacte*r virulence (*cdt*ABC complex, *cia*B and virB11) were investigated in *C. coli* strains. *cia*B polymorphisms found in the food chain strains suggested that infective strains could be from a source other than chicken meat. Chicken strains clustered in a unique clone after MLST+flaA typing of *C. coli* strains. Data suggest that a significant proportion of human isolates might be considered of no-chicken origin.

Conclusions

C. coli MRD-EryR isolated from humans with no-chicken origin could be an emerging risk for the European consumer.

FEMS7-0550
Food Microbiology - Part II

ISOLATION AND CHARACTERIZATION OF QUAMBALARIA CYANESCENS FROM UNRIPENNED GRAPES.

J.J. Mateo Tolosa[1], *S. Maicas*[1], *F. Rosa*[1], *Z. Raquel*[1]
[1]*Universitat de Valencia, Microbiología y Ecología, Valencia- Burjassot, Spain*

Backgrounds

Quambalaria cyanescens is a hyaline basidiomycete isolated from a broad range of ecological niches, including air, soil, and insect larvae as well as in association with diverse plant sources in various countries. *Q. cyanescens* is frequently associated with bark beetles feeding on numerous plants. This fungus, however, is predominantly known as non-pathogenic to plants and has been reported as a symbiont of plants, including *Corymbia* and *Eucalyptus* species, but it has never been isolated from grapes.

Objectives

The aim of this work was to isolate, identify and characterize the fungus *Quambalaria cyanescens* from unripenned grapes.

Methods

Q. cyanescens was isolated on Malt Agar plates and were analysed by RFLP analysis of the 5.8S-ITS rDNA region. For sequence analysis of the D1/D2 domains of the 26S rDNA gene, PCR amplification was performed Physiologically characterization was developed in both API ZYM and API C AUX strips and also in specifc plates.

Conclusions

Q.*cyanescens* was positively identified on the surface of unripenned grapes. Some of the isolates show different enzymatic activities with potential biotechnological interest.

This work was supported by the project “Identification and biotechnological characterization of yeasts isolated from agrifood residues of the Valencian Community”, grant AICO-2016-079.

FEMS7-1788
Food Microbiology - Part II

BACTERIAL ECOLOGY OF BLACK RIPE OLIVE PROCESSING

E. Medina[1], *C. Romero*[1], *M. Brenes*[1], *P. García*[1], *A. de Castro*[1]
[1]*Instiuto de la Grasa - CSIC, Food Biotechnology, sevilla, Spain*

Backgrounds

Black ripe olives are one of the two most important types of table olives. However, the microbiology during their production is lacking.

Objectives

The objective of this research was to characterize the biochemistry and bacterial biodiversity along the different stages of the black ripe olive processing.

Methods

The research was carried out on Manzanilla and Hojiblanca cultivar samples from a leading table olive factory.

Conclusions

Biochemical analysis showed a high amount of total sugar and polyphenol concentrations in the preservation first stage (43.32 mmoles/Kg and 15.60 mM, respectively). These compounds decreased along the processing to 1.41 mmoles/Kg and 2.54 mM at the final wash before packing. High populations of lactic acid bacteria (LAB), yeast and moulds, and *Enterobacteriaceae* were differentially detected by using culture-dependent techniques.

The novelty of this research was the study of the microbial ecology of black ripe olive processing using high-throughput DNA sequencing of 16s rDNA. During preservation, first wash, and ferrous gluconate stages, the predominant genera were *Lactobacillus* and *Acetobacter*. The microbiota showed a greater biodiversity at the last two phases of the process, and other microorganisms were found, as other LAB genera (*Enterococcus*, *Leuconostoc*, *Weissella*, *Lactococcus*, *Streptococcus*). Besides, this is the first time that genera such as *Shewanella*, *Marinomonas*, *Alteromonas*, *Acinetobacter*, and *Vibrio*, among others, were identified in this type of table olive processing. Although black ripe olives are sterilized before commercialization, this study will contribute to the knowledge of the bacterial communities present in this product and their overall significance on the final quality.

FEMS7-0947
Food Microbiology - Part II

EVALUATION OF THE DIFFERENT SALMONELLA SEROTYPES ISOLATED FROM FARS PROVINCE POULTRY FARMS TO SENSITIVITY ANTIBIOTIC ASSAY DISC

M.J. Mehrabanpour[1], E. Rahimi larki[2], S. Moradi Bidhendi[3], D. Mehrabanpour[4]
[1]Razi vaccine and serum research institute- Shiraz branch-
Agricultural Research- Education and Extension Organization AREEO-Shiraz-Iran, virology, Shiraz, Iran
[2]Razi vaccine and serum research institute- Shiraz branch, Microbiology, Shiraz, Iran
[3]Razi vaccine and serum research institute-Hesark-karaj, Bactriology, karaj, Iran
[4]Islamic Azad Universty- colleage of veterinary-Kazeroun-Iran, Microbiology, Kazeroun, Iran

Backgrounds

Salmonella spp. is a group of gram negative bacillus bacteria which should be considered as an important zoonotic disease including poultry and their products. This bacterium is scattered all around the world from Asia to America and Africa. It is recently shown that poultry, meat products and eggs which had been infected or contaminated by those bacteria are the main source of contamination. Therefore, prevention and killing salmonella in source should be mentioned.

Objectives

The aim of this study is evaluating the antibiotic sensitivity of different salmonella serotypes isolated from Fars province poultry farms.

Methods

For this reason, 713 cloacal swab samples from 25 poultry farms were collected. Then all the microbiological examinations on the samples were done. Six of them were suspected for salmonella infection and with specific examinations; two samples with *Salmonella Enteritidis* and one sample with *Salmonella Thyphymurium* were accurately detected. Finally, the antibiotic susceptibility of them was evaluated using antibiotic assay discs (disc diffusion).

Conclusions

The results were shown that all the isolated bacteria were sensitive to Ciprofloxacin, Tetracycline, Gentamycin, Kanamycin and Nalidixic acid. But, they had different reactions to other antibiotics. It seems that antibiotic resistance has been emerged between salmonella serotypes, even in the various isolate from one serotype.

FEMS7-2381
Food Microbiology - Part II

MOLECULAR IDENTIFICATION AND ANTIMICROBIAL RESISTANCE OF SALMONELLA SEROTYPES IN MEAT PRODUCTS FROM ECUADOR

L. Mejia Castaneda[1], *G. Vela*[1], *A.L. Freire Vélez*[1], *S. Zapata Mena*[1]
[1]*Universidad San Francisco de Quito, Institute of Microbiology, Cumbayá, Ecuador*

Backgrounds

Salmonella is responsible for foodborne infections worldwide and it is one of the four global causes of diarrhoeal diseases. It is generally transmitted by the consumption of contaminated food from animal products. On the other hand, antimicrobial resistance is one of the most important topics on public health. Also, some multidrug resistant Salmonella serotypes have emerged in different parts of the world.

Objectives

The aim of this study was to determine the prevalence and antimicrobial resistance of Salmonella serotypes isolated from meat products in Ecuador.

Methods

Sample collecting was done during 2015 and the microbial analysis was done with 3M Molecular Detection Assay Salmonella ® and by McCormick Method M0740 for isolation of Salmonella. Serotypes were determined by multiplex PCR. Antimicrobial resistance patterns for 14 antibiotics (CLSI recommendations) were obtained by disk diffusion assay-Kirby-Bauer method.

Conclusions

A total of 580 samples were collected. The prevalence for Salmonella was 43,10%. For the aim of this study, 70 isolates and 130 lysates corresponding to 140 samples were further analized. The most common serotype was Infantis (79,3%), followed by Gallinarum (15%). Typhimurium and Cholera suis were found in 2,14% of the samples while Enteritidis and Dublin in only 1,42%. All isolates were sensitive to Amoxicillin/clavulanate and azithromycin but most (more than 80%) were resistant to tetracycline, streptomycin, nalidixic acid, cefotaxime, ciprofloxacin, gentamicin, ampicillin and ceftriaxone. Most of the isolated strains showed high resistance patterns affecting the food chain in Ecuador. This is the first study on serotype identification and antimicrobial resistance on Salmonella from meat products.

FEMS7-0952
Food Microbiology - Part II

BACTERIAL COMMUNITIES IN BOVINE MILK DURING PROCESSING AND STORAGE

D. Porcellato[1], M. Aspholm[2], S.B. Skeie[1], M. Monshaugen[2], J. Brendehaug[3], H. Mellegaard[2]
[1]Norwegian University of Life Sciences, Faculty of Chemistry- Biotechnology and Food Science, Ås, Norway
[2]Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Oslo, Norway
[3]TINE SA, R&D, Stavanger, Norway

Backgrounds

Bovine milk is a valuable nutriment worldwide. It contains a complex microbial community, which may affect the quality and safety of the product. Detailed knowledge of this microbiota during processing and storage is therefore of importance for the dairy industry in their quality and safety assurance work.

Objectives

Our goal was to characterize the bovine milk microbiota, also capturing seasonal variations, from raw to processed milk at the dairy plant, and throughout the shelf life period of full fat milk cartons.

Methods

Monthly samples from two Norwegian dairy plants, differing in their production volume, were collected over a 13 months period. 16S rRNA marker gene sequencing and standard culturing techniques were applied to study the bacterial composition during processing, from raw milk to full fat milk during processing at the dairy plant, and during product storage at 4 °C and 8 °C, where the latter temperature simulate suboptimal storage conditions.

Conclusions

The examined milk samples contained a high bacterial diversity where sampling month, processing stage and storage temperature had a significantly impact on the level and composition of the bacterial community. Especially, storage at 8 °C resulted in a significant increase in the level of *Bacillus* spp. compared to storage at 4 °C. Despite this variation, a core microbiota was identified in samples from both dairies. This large-scale study provide detailed insight into the dynamics of the bacterial community along the milk value chain.

FEMS7-0040
Food Microbiology - Part II

ENTEROTOXIGENIC STAPHYLOCCOCUS AUREUS IN BRINED CHEESE FROM WEEKLY MARKETS IN TURKEY

B. Mercanoglu Taban[1], S. Karimihachehsoo[1], Ö. Akineden[2], M. Gross[3], E. Usleber[2]
[1]Ankara University- Faculty of Agriculture, Dairy Technology Department, Ankara, Turkey
[2]Institute of Veterinary Food Sciences- Justus-Liebig-University Giessen, Dairy Sciences, Giessen, Germany
[3]Institute of Veterinary Food Sciences- Justus-Liebig University Giessen, Junior Professorship of Veterinary Food Diagnostics, Giessen, Germany

Backgrounds

Traditionally produced brined cheeses (White pickled cheese, Tulum cheese) are frequently offered as unpacked local produce on weekly markets in Turkey, and are both very popular and economically important products.

Objectives

In this study, *Staphylococcus* (*S*.) *aureus* was determined in a total of 63 unpacked brined cheeses from weekly markets in the Turkish province of Ankara. Isolates from positive samples were analyzed for staphylococcal enterotoxin (SE) genes by PCR, and for SEA-SEE by ELISA.

Methods

S. aureus was found in 11 (17.0%) cheeses (8/33 Tulum cheeses, 3/30 other white pickled cheeses), the highest contamination was 5.0 x 10^6 cfu/g. All 22 tested isolates from these samples were positive for SE genes. By ELISA, several isolates from Tulum cheeses produced SEA or SED. By PCR, the corresponding genes (*sea*, *sed*) were found in these isolates. Further, many trans-SE genes (*seg*, *sei*, *selj*, *sem*, *sen*, *seo*, *sep*, *ser,* and *selu*), but only three different gene combinations, were detected by PCR in isolates from both types of cheeses. Likewise, further characterization of the 22 isolates by macrorestriction analysis yielded three different pulsed-field gel electrophoresis (PFGE) profiles only, which corresponded well with the three SE gene profiles (*sea*+*sed*+*selj*; *sed*+*ser*; *seg*, *sei*, *sem*, *sen*, *seo*, *sep*, *selu*).

Conclusions

This contamination pattern indicates that all 11 *S. aureus*-positive cheeses, which had each been sold as “unique produce” by different sellers, in fact originated from three sources only, and thus did not comply with labelling requirement. This also shows the strength of PFGE for contamination-based fingerprinting of artisanal food products relatively high frequency and levels of enterotoxigenic *S. aureus*. In conclusion, the findings presented here demonstrate the need of a more intensive and efficient hygiene control of unpackaged, traditionally produced brined cheeses to avoid health hazards for food safety.

FEMS7-1365
Food Microbiology - Part II

PHAGE HOST-ADAPTATION IMPROVES BIOCONTROL IN CHEESEMAKING AND WIDENS THE LYSIS SPECTRUM OF ENTEROBACTERIA

F. Molina[1], A. Simancas[1], R. Tabla[2], I. Roa[2], J.E. Rebollo[1]
[1]Universidad de Extremadura, Genetics, Badajoz, Spain
[2]Technological institute of Food and Agriculture, Dairy products, Badajoz, Spain

Backgrounds

Bacterial contamination in food production suppose both a health issue and an economic loss for food manufacturers. In dairy industry, *Escherichia coli* strains coming from animal stool have been identified as a threat, as they spoil cheese-making processes, thus rendering a final product not suitable for human consumption. Along with *E. coli*, other *Enterobacteria* species have been identified in cheese spoilage, being some *Shigella* strains among them. Due to the high molecular similarity between *E. coli* and these *Shigella* strains, we looked towards obtaining a phage therapy treatment involving the use of viruses able to infect both bacterial genus.

Objectives

Based on lysis spectrum analysis results, we wanted to enhance crossed infection activity of bacteriophages isolated on *E. coli* against *Shigella* strains in order to obtain a phage cocktail suitable for a wider range of bacterial targets in dairy industry biocontrol.

Methods

Bacteriophages were isolated for local sheep farms and tested with an *Enterobacteriaceae* lab collection and cheese contaminant strains. After screening the two most efficient viruses against both E. coli and several *Shigella* strains were selected. *Shigella* strains were infected in consecutive five-hours infection cycles, up to a total of 80 cumulative hours. Assays were then conducted to measure relative viral particles production, plaque size and both bacterial lysis and growth inhibition.

Conclusions

Local adaptation assays proved to be a useful way to improve the host range of our bacteriophages, hence leading to an improvement on the phage therapy treatments used on dairy industry.

FEMS7-1585
Food Microbiology - Part II

VALIDATION OF A SYBR GREEN REAL TIME PCR ASSAY FOR RAPID ENUMERATION OF SALMONELLA SPP.

S. Momtaz[1], J. Mandal[1], M.A. Siddique[1], M. Sultana[1], M.A. Hossain[1]
[1]University of Dhaka, Microbiology, Dhaka, Bangladesh

Backgrounds

Salmonellosis, a major food borne infection in human, is caused by *Salmonella* spp. and has become a prominent public health concern in Bangladesh.

Objectives

The classical detection techniques for *Salmonella* are limited due to time consumption and requirements of series of tests for confirmation. This study was designed to develop a rapid, reproducible, and robust method for detection and quantification of *Salmonella* spp. based on Quantitative real-time PCR (qPCR).

Methods

qPCR corresponds to absolute copy number for a particular target gene directly implicating the cell numbers. Therefore, a gold standard of template implicating in direct quantification of the cell number is required. In this study, copy number of *Salmonella* spp. specific gene *invA* was considered. Three types of template standards were used such as purified *invA* amplicon products of 284 bp, cloned *inv*A amplicon (284bp) in the TOPO TA vector and *Salmonella* Enteritidis IFO 3313 gDNA. Suitable DNA extraction methods were chosen among Phenol Chloroform DNA extraction method, boiling method, automated DNA extraction method (Maxwell® 16 DNA extraction system,) and commercial kit based method such as solution based kit (Jena Bioscience GmbH, Germany) and column based kit (ATP Biotech Inc, USA). The specificity of the reaction was confirmed by the melting temperature (T_m) of the amplicon obtained.

Conclusions

Findings of this study demonstrate that recombinant plasmid DNA (cloned *inv*A amplicon) based standard curve, phenol Chloroform DNA extraction method and SYBR Green I Real-time PCR constitutes an effective and easy-to-perform method for detecting *Salmonella* spp.

FEMS7-0153
Food Microbiology - Part II

THE FIRST DETECTION OF FLORFENICOL RESISTANT GENE IN SHIGA-LIKE TOXIN PRODUCING ESCHERICHIA COLI ISOLATED FROM PORK IN KOREA

J.S. Moon[1]
[1]*Animal and plant quarantine agency, veterinary pharmaceutical management, Gimcheon-si, Republic of Korea*

Backgrounds

Florfenicol is fluorinated derivative of chloramphenicol and represent highly protein inhibitors of bacterial protein biosynthesis. It is used in veterinary medicine or feed additives for pigs in Korea. The transfer of antibiotic resistance gene among the bacterial strains has become a problem worldwide, so drug resistant bacterial phenotypes should be identified.

Objectives

In this study, antimicrobial resistance and resistance gene were investigated for shiga-like toxin producing *E. coli (*STEC) isolated from pork in Korea.

Methods

We monitored 301 pork samples in slaughter houses and retail markets, and isolated 50 strains of *E. coli* in 2013. Among these isolates, six isolates resulted in STEC. Minimum inhibitory concentration (MIC) on six strains was performed for 14 antibiotics, ampicillin, amoxicillin/clavulanic Acid, cefoxitin, ceftiofur, cephalothin, florfenicol, ciprofloxacin, colistin, gentamicin, nalidixic acid, neomycin, streptomycin, tetracycline and trimethoprim/Sulphamethoxazole, and three strains showed high MIC to florfenicol and chloramphenicol (64 ug/mL). PCR was conducted to detect the florfenicol resistant gene (*floR*) and the chloramphenicol resistant gene (*cat*).

Conclusions

All of 3 strains contained the *floR*, while none of them had the *cat*. These PCR products were sequenced and aligned to obtain homology with other available genes in reference GenBank. A BLAST search showed that they contained sequences with homology to the *floR* gene of *E. coli* or *Salmonella enteric* serovar Heidelberg.This is the first report to detect *floR* gene in STEC isolated from slaughtered pigs in Korea. These results suggest that some STEC isolates in Korea carry florfenicol resistant gene and transfer this gene to other bacterial strains.

FEMS7-0154
Food Microbiology - Part II

PREDICTIVE MODEL FOR SALMONELLA GROWTH IN UNPASTEURIZED LIQUID WHOLE EGG, EGG YOLK AND EGG WHITE IN KOREA

J.S. Moon[1]
[1]*Animal and plant quarantine agency, veterinary pharmaceutical management, Gimcheon-si, Republic of Korea*

Backgrounds

There are concerns on the quality of non-pasteurized liquid eggs depending on storage temperature and time because they are processed without heat-treatment and marketed.

Objectives

The objective of this study was to determine the risk of *Salmonella* by investigating the growth rate according to the time of various storage temperatures of the unpasteurized liquid whole egg, egg yolk, and egg white.

Methods

Samples were inoculated with a mixed culture (approximately 3.0 log CFU/ml) containing equal proportions of five strain cocktail of *Salmonella* (*S.* Bareilly, *S.* Richmond, *S.* Typhimurium monophasic, *S*. Enteritidis and *S*. Gallinarum) egg isolates. After inoculating *Salmonella* spp. onto samples, the effects of storage temperatures (5-40℃) on the growth of *Salmonella* spp. were investigated by the 960 hours.

Conclusions

The results showed that the population of *Salmonella* in liquid whole egg and yolk increased at over 10℃, while *Salmonella spp*. in egg white did not grow at any temperature. Growth curves of *Salmonella* in liquid whole egg and yolk were fitted to the Baranyi model, and lag phase duration (LPD) and maximum specific growth rate (μ_{max}) were obtained. LPD significantly ($p<0.05$) decreased with a storage temperature and it were higher in egg yolk than liquid whole egg. μ_{max} significantly ($p<0.05$) increased as the storage temperature increased. The secondary model, verified as suitable model, can be used for predicting the growth of *Salmonella spp*. in liquid whole egg and egg yolk under a variety of storage temperature and time conditions.

FEMS7-0852
Food Microbiology - Part II

OPTIMIZATION OF PARAMETERS FOR ETHANOL REDUCTION IN WINE BY RESPIRATION

M. Callerand[1], R. Gonzalez[1], P. Morales[1]
[1]ICVV CSIC-Universidad de La Rioja-Gobierno de La Rioja, Enología, Logroño, Spain

Backgrounds

The increase in alcohol levels in wine during the last decades is a main concern for the wine industry. One of the solutions under study consists in the use of non-*Saccharomyces* strains in aeration condition to respire the excess of sugars. Some species of non-*Saccharomyces* have been identified as good candidates for this purpose, but a fine tuning of conditions is required to get a quality product.

Objectives

In this work, the conditions for the co-culture of *S. cerevisiae* and *C. sake* with the aim to reduce alcohol levels of wine were investigated.

Methods

Three parameters, the moment of addition of *S. cerevisiae*, the amount of *S. cerevisiae* inoculum, and the amount of air used during aeration period were studied. Three levels of each parameter were combined in a Box-Behnken design, and modeling of the effect of these parameters on metabolite production and sugar consumption was obtained after statistical analysis of experimental data.

Conclusions

The glycerol yield and sugars consumed during aeration were dependent on the three variables studied, while ethanol yield was not dependent of the amount of *S. cerevisiae* used. Conditions for maximal sugar consumption were close to the central point of the experiment. Model equations were:

Ethanol Yield =0.349 -0.018*TSc - 0.026*Air +0.018*TSc ^{2}

Glycerol Yield =0.032 -0.003*Air +0.004*Tsc *[Sc] +0.003 *Air^{2}

Consumed sugars in day 2 =56.460 –2.979*TSc*[Sc] -3.598*TSc^{2} -4.474*[Sc]^{2} -4.982*Air^{2}

Acknowledgments: MINECO(AGL2015-63629-R, RTC-2014-2186-2) EU(7FP-IRSES-GA612441)

FEMS7-1271
Food Microbiology - Part II

SCALING-UP AERATED FERMENTATION FOR ETHANOL REDUCTION. EFFECT ON WINE AROMA

J.A. Curiel[1], J. Tronchoni[1], M.P. Sáenz-Navajas[2], A. de la Fuente[2], P. Fernández-Zurbano[1], V. Ferreira[2], P. Morales[1], R. Gonzalez[1]
[1]ICVV CSIC-Universidad de La Rioja-Gobierno de La Rioja, Enología, Logroño, Spain
[2]Instituto Agroalimentario de Aragón IA2- Universidad de Zaragoza,
Laboratory for Flavor Analysis and Enology-LAAE, Zaragoza, Spain

Backgrounds

The process for ethanol reduction by using aerated fermentation conditions, and a combination of *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* as starter cultures, has been previously optimized at laboratory scale.

Objectives

Since previous trials indicated a limitation of non-*Saccharomyces* strains in a non-sterile, sulfite treated, natural grape must, the objective of this work was scaling-up the process to 20 L vats. In addition, we wanted to minimize acetic acid production, also identified as a potential drawback of oxygenated fermentation.

Methods

Dominance was reached by preadaptation of non-*Saccharomyces* strains to grape must conditions and relatively high inoculation titles. The other big challenge has been to change the automatic control of aeration based on dissolved oxygen detection (as used in small scale bioreactors) to a controlled but constant aerated period (more amenable to industrial application).

Conclusions

Wines made with *M. pulcherrima* or *Torulaspora delbruekii* under aeration, followed by a final step with *S. cerevisiae* under standard conditions, contained less alcohol than the control wines (*S. cerevisiae* under standard conditions). Acetic acid levels were not increased by aeration.

A classification based on aroma of replicates of wines by a trained panel, divided wines by strain. *S. cerevisiae* wines were linked to white fruit/pear and tropical fruit/banana aroma descriptors; while *T. delbruekii* wines were linked to dried fruit/nuts and reduction descriptors; and *M. pulcherrima* wines were linked to oxidation/spirits-like descriptors. The later can be explained by the higher amount of isoamyl and isobutyl alcohols and ethyl acetate found in these wines.

Acknowledgments: MINECO (AGL2015-63629-R, RTC-2014-2186-2) EU(7FP-IRSES-GA612441)

FEMS7-1163
Food Microbiology - Part II

AMINO ACID METABOLISM DURING THE SECOND FERMENTATION IN THE ELABORATION OF CAVA BY TWO SACCHAROMYCES CEREVISIAE WINE STRAINS: A FIRST PROTEOMIC APPROACH

M.D.C. Gonzalez Jiménez[1], J. Moreno García[2], J.A. Porras Agüera[1], F. Capdevila Mestres[3], A. Puig Pujol[3], J.J. Moreno Vigara[4], J.C. García Mauricio[1], T. García Martínez[1]
[1]University of Córdoba, Microbiology, Córdoba, Spain
[2]University of Córdoba, Microbiology, Cordoba, Spain
[3]IRTA-INCAVI, Oenological Research, Vilafranca del Penedès, Spain
[4]University of Córdoba, Agricultural Chemistry, Córdoba, Spain

Backgrounds

Cava is a sparkling wine characterized by the occurrence of two consecutive fermentations in its elaboration process: a first fermentation, where the base wine is obtained; and a second fermentation, which occurs in a closed bottle. During the second fermentation carbonic gas, characteristic of this type of wine, is originated. The main nitrogen source for yeasts involved in this process are amino acids, which metabolism can contribute to the aroma and the flavor of the obtained wines.

Objectives

The objective of this work is to compare the amino acid metabolism through a proteomic approach in the middle of the second fermentation in closed bottle in two different yeast wine strains of *Saccharomyces cerevisiae*: a traditionally used strain used for sparkling wine elaboration and a flor yeast strain commonly used for the elaboration of sherry wines which is also able to survive after the fermentation.

Methods

To carry out the proteomic analysis cells were broken, and extracted proteins were separated using OFFGEL protein fractionation, LTQ Orbitrap MS analysis. For the treatment and discussion of results, the *Saccharomyces* Genome Database was used.

Conclusions

A total of 113 proteins were identified for the sparkling wine strain, from which 28 were specific, 8 proteins involved in the amino acid biosynthesis and 6 proteins in the amino acid catabolism pathways. 108 proteins were obtained in the flor yeast, resulting 23 to be specific to this strain. In this case, 10 proteins related to amino acid synthesis pathways and 6 proteins in catabolic pathways were identified.

FEMS7-0188
Food Microbiology - Part II

INTEGRATED TREATMENT: A SENSIBLE APPROACH TO PRODUCE SAFETY, QUALITY AND SUSTAINABILITY

S. Mukhopadhyay[1]
[1]*United States Department of Agriculture, Agricultural Research Service, Wyndmoor, USA*

Backgrounds

Current chlorine based washing method for fresh produce has limited effectiveness and poses serious public health concern.

Objectives

The purpose of present study was to investigate the efficacy of different combined non-thermal treatments against food borne pathogens and natural spoilage organisms.

Methods

Smooth surface and stem scars of tomato and spinach leaves inoculated with a bacterial cocktail containing a three serotype mixture of *S. enterica* and a three strain mixture of *E. coli* O157:H7 were subjected to different integrated treatments. Treatment combining UV-C light (0.6 kJ/sq. m) and wash in mixed acid (1% lactic plus citric acid) for 2 min provided 3.9 ± 0.6 log CFU/fruit reduction for *Salmonella* on tomato compared to 2.6 ± 0.4 log CFU/g reduction for *E. coli* on spinach leaves. A combination treatment of 0.6 kJ/sq. m UV-C followed by 2 min wash in a novel antimicrobial solution of HEN (mixture of H_2O_2, EDTA and Nisin) provided synergistic inactivation of 4.71 ± 0.25 logs for *Salmonella* on tomato which is superior to current chlorine based decontamination practice providing only 2.4 ± 0.37 logs reduction. These treatments also significantly ($p < 0.05$) reduced populations of native aerobic plate count, and yeasts and molds of tomato fruits and spinach leaves. Furthermore, firmness and color of samples were not significantly affected by the treatment.

Conclusions

Results indicated that the efficacy of the integrated treatment depended on types of inoculated bacteria and produce, and the technology presented here could potentially be used as a replacement for current chlorine based produce sanitizing practice.

FEMS7-0680
Food Microbiology - Part II

SAFETY RISKS LINKED TO HOME SPROUTING MODULES

E. Mulaosmanovic[1], S. Farkas[1], J. Linden[1], M. Sousa[1], S. Gharaie[1], L. Mogren[1], I. Vågsholm[2], B. W. Alsanius[1]
[1]Swedish University of Agricultural Sciences SLU, Department of Biosystems and Technology-Microbial Horticulture Unit, Alnarp, Sweden
[2]Swedish University of Agricultural Sciences SLU,
Department of Biomedical Science and Veterinary Public Health-Division of Bacteriology and Food Safety, Uppsala, Sweden

Backgrounds

Escherichia coli O157:H7, a hazardous foodborne pathogen, has been in the spotlight several times during the last decades due to its infectivity and epidemic nature of dispersal. Recent outbreaks of *E. coli* infections associated with consumption of raw vegetables, predominantly sprouted seeds, have been reported in several countries around the world. At the same time growing and raw consumption of sprouted seeds is associated with a healthy lifestyle in Scandinavia, and different types of sprouting modules for home production are available. During sprouting ideal conditions for emergence, but also for bacterial proliferation are prevailing. As the seeds are non-sterile, also human pathogens may propagate, which poses a great risk of foodborne illness.

Objectives

We studied the dynamics of *E. coli* O157:H7 *gfp+* in two types of three-layered sprouting modules, using organic fenugreek seeds (*Trigonella foenum-graecum*) as a model crop.

Methods

E. coli O157:H7 *gfp+* was introduced to either bottom or top layer of the modules and its dispersal was analyzed after 3.5 days. Using Beta-Poisson model we assessed the risk for consumer exposure.

Conclusions

We found that the inoculated strain dispersed both vertically up and down between the layers. Our results indicate that risk for dispersal of *E. coli* O157:H7 is high in both directions within the sprouting module, posing a high risk for consumer exposure. Data on the bacterial background biota is presented in the poster. We conclude that high hygiene standards are needed for both seeds and modules used for home sprouting of seeds.

FEMS7-2248
Food Microbiology - Part II

THE ROLE OF NITROGEN UPTAKE ON COLONIZATION ABILITY OF AUTOCHTHONOUS SACCHAROMYCES CEREVISIAE STRAINS

C. Vendramini[1], G. Beltran[2], C. Nadal[1], G. Crosato[1], A. Giacomini[1,3], A. Mas[2], V. Corich[1,3]
[1]University of Padova, DAFNAE, Legnaro, Italy
[2]Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Tarragona, Spain
[3]University of Padova, CIRVE, Conegliano, Italy

Backgrounds

Saccharomyces cerevisiae is the main actor of the alcoholic fermentation and the use of selected strains as starter cultures to initiate and control alcoholic fermentation nowadays is a worldwide practice. However, produced wines are standardized and with poor complexity. For this reason, a wide pool of autochthonous yeasts was isolated from grape bunches coming from vineyards of different winemaking regions in the North-East of Italy. Their fermentation performances were studied to select strains for the production of wines with enriched complexity.

Objectives

The aim of this work was to understand if nitrogen availability could influence vineyard strain colonization ability during must fermentation.

Methods

Selected vineyard strains of *Saccharomyces cerevisiae* were assayed in competence with a commercial industrial strain. Pairwise-strain fermentations and co-fermentations were performed in synthetic musts at two nitrogen levels: control nitrogen condition (CNC), assuring the suitable assimilable nitrogen amount required by the yeast strains to complete the fermentation, and low nitrogen condition (LNC), where nitrogen is present at very low level. Single-strain fermentations were also run in both conditions and the nitrogen metabolism analyzed.

Conclusions

The high nitrogen assimilation rate seems to be an additional strategy that allowed vineyard yeasts successful competition during must colonization.

FEMS7-3179
Food Microbiology - Part II

PENICILLIUM ROQUEFORTI DEVELOPMENT DURING SWEET GORGONZOLA CHEESE PRODUCTION

E. Neri[1], S. Arioli[1], S. Colombo[1], G. Della Scala[1], G. Gargari[1], M. Stuknyte[2], I. De Noni[2], F. Dal Bello[3], A. Bernacchi[3], E. Nicita[4], D. Mora[1]
[1]University of Milan, Department of Food- Environmental and Nutritional Sciences DeFENS, Milan, Italy
[2]University of Milan, Department of Biochemistry, Milan, Italy
[3]Sacco SRL, Research & Development, Cadorago, Italy
[4]Caseificio fratelli Oioli, Quality Control, Cavaglietto, Italy

Backgrounds

Sweet gorgonzola is a blue-veined, mould-ripened cheese, made from pasteurized cow's milk inoculated with starter cultures of lactic acid bacteria along with *Saccharomyces cerevisiae* and *Penicillium roqueforti.* Fungal growth during the ripening process is crucial for the final quality of the cheese. Traditional microbiological methods along with alternatives (ergosterol, dry weight, spores counting) are not suitable for hyphal filaments quantification in the cheese matrix.

Objectives

Here we monitored and compared the *P. roqueforti* development in two Gorgonzola productions during all the steps of the cheese production process.

Methods

We developed a qPCR assay based on a species-specific primer set targeted on *ari1* gene. The development of *P. roqueforti* in the cheese matrix was compared with data related to cheese proteolysis obtained with a mass spectrometry-based chemical analysis and using a glutamate-sensitive *S. thermophilus* luminescent biosensor.

Conclusions

The results obtained showed a good agreement between the proteolysis dynamics, measured during cheese ripening, and the qPCR data. The overall data obtained underlined that the production of sweet Gorgonzola is characterized by high degree of variability related to the growth of *P. roqueforti* within the cheese matrix. A limited growth of the mould is reflected in a limited proteolysis with a consequent reduction of creaminess and cheese sensory properties. Sugar consumption measurements during the first stages of cheese production led to hypothesize that the development of *P. roqueforti* during cheese ripening is strictly dependent on the appropriate growth and galactose fermenting capability of *Saccharomyces cerevisiae* during the first 36 h of Gorgonzola production process.

FEMS7-2859
Food Microbiology - Part II

PREBIOTIC EFFECT OF XYLOOLIGOSACCHARIDES PRODUCED FROM BIRCHWOOD XYLAN BY A NOVEL FUNGAL GH11 XYLANASE

M. Nieto-Domínguez[1], L.I. de Eugenio[1], M.J. York-Durán[2], B. Rodríguez-Colinas[2], F.J. Plou[2], E. Chenoll[3], E. Pardo[3], F. Codoñer[4], M.J. Martínez[1]
[1]Centro de Investigaciones Biológicas CIB-CSIC, Environmental Biology, Madrid, Spain
[2]Instituto de Catálisis y Petroleoquímica ICP-CSIC, Biocatálisis Aplicada, Madrid, Spain
[3]Biopolis S.L., Microbiology, Valencia, Spain
[4]Lifesequencing S.L., Lifesequencing, Valencia, Spain

Backgrounds

Xylooligosaccharides (XOS) are nutraceutics derived from xylan hydrolysis, which are receiving increasing attention since their potential as emerging prebiotics is becoming evident. These carbohydrates are composed of β-1,4-linked xylopyranose residues. XOS have demonstrated their capacity to selectively stimulate the growth of probiotic microorganisms present in the lower gastrointestinal tract, as *Lactobacillus* and *Bifidobacterium* species. They are obtained from the xylan fraction of lignocellulosic materials by physicochemical or enzymatical methods. The latter displayed the advantages of being environment-friendly, requiring mild conditions, and producing low monosaccharide yield without toxic by-products.

Objectives

The main goals of this work were the application of a novel GH11 endoxylanase, isolated from the ascomycete *Talaromyces amestolkiae*, to convert birchwood xylan into XOS, the characterization of the obtained mixture, and the assay of its prebiotic potential.

Methods

XOS were produced using the purified endoxylanase in its optimal reaction conditions. The production yields were evaluated by HPLC (HPAEC-PAD), while the polymerization degrees' distribution and the composition of the mixture were assessed by HPAEC-PAD and ESI-MS. Biological activity of the obtained XOS mixture was evaluated by fecal-fermentation, determining the profiles of organic acids and analyzing bacterial microbiomes up to the species level.

Conclusions

By this way a mixture of birchwood XOS including neutral and charged species was obtained in a yield comparable to other reports, with negligible content of xylose. These oligosaccharides displayed prebiotic properties, promoting the growth of bifidobacteria together with the potential probiotic strain *Staphylococcus hominis*.

Thanks to MINECO BIO2015-68387-R and CM S2013/MAE-2907 for funding.

FEMS7-1672
Food Microbiology - Part II

TRANSCRIPTIONAL AND TRANSLATIONAL CHANGEOVER DURING B. SUBTILIS SPORE GERMINATION

B.N. Swarge[1], D. Beaty[1], W. Roseboom[2], S. Brul[3], C.G. de Koster[2], L.J. de Koning[2]
[1]Swammerdam Institute for Life Sciences- University of Amsterdam,
Molecular biology and Microbial Food Safety- Mass spectrometry of Biomacromolecules, Amsterdam, The Netherlands
[2]Swammerdam Institute for Life Sciences- University of Amsterdam,
Mass spectrometry of Biomacromolecules, Amsterdam, The Netherlands
[3]Swammerdam Institute for Life Sciences- University of Amsterdam,
Molecular biology and Microbial Food Safety, Amsterdam, The Netherlands

Backgrounds

In response to nutrient limitation Gram positive organisms like *Bacillus subtilis* form dormant spores. These cellular entities are survival capsules, resistant to chemical and environmental assaults. They pose challenges to the food and medical sectors. Upon contact with germinants spores return to vegetative life through a process called 'germination and outgrowth'. Spores reactivate their metabolism and develop in vegetative cells. The molecular machinery that triggers and progresses the germination is still unsettled.

Objectives

Gain insight in the germination molecular machinery by mapping the progression of the transcriptome and proteome changeover during germination and outgrowth of spores.

Methods

For the time-resolved monitoring of the transcriptome and the proteome *of B. subtilis spores* during germination, the spores are sampled in time-intervals from germination initiation to the stage of vegetative cell outgrowth. Intact and purified RNA is isolated from spores, at each time-point, for transcriptome analysis. We thus trace the different functional groups of genes expressed during the germination and outgrowth process. Next, the changes in the proteome are quantitatively monitored relative to the proteomes of ^{15}N metabolically labelled dormant spores and ^{15}N metabolically labelled vegetative cells.

Conclusions

The time-resolved transcriptomics and proteomics data provide new insight in the cellular control level of novel protein synthesis during the awakening of spores. The data will offer input for the development of a mathematical model of the germination process.

FEMS7-2703
Food Microbiology - Part III

PREVALENCE OF NON-TUBERCULOUS MYCOBACTERIA WITH VIRULENCE PROPERTIES ISOLATED FROM RAW MILK AND STREET-VENDED DAIRY PRODUCTS

D.E. Rios-Muñiz[1], J.F. Cerna Cortesa[1], E.B. Angeles-Morales[1], M. Bodadilla-del Valle[2], A. Ponce-de Leon[2], M.T. Estrada-Garcia[3]
[1]Instituto Politécnico Nacional, Microbiology, Mexico City, Mexico
[2]National Institute of Medical Science and Nutrition, Infections Diseases, Mexico City, Mexico
[3]CINVESTAV-IPN, Molecular Biomedicine, Mexico City, Mexico

Backgrounds

Reports of human infectious and illness due to non-tuberculous mycobacteria (NTM) and NTM isolation from a variety of food samples have recently increased worldwide. Biofilm formation and sliding motility are characteristics that allow NTM to persist on human tissues and natural reservoirs.

Objectives

To establish NTM prevalence in dairy products and raw milk and the antibiotic susceptibility, biofilm formation and sliding motility of the NTM isolates

Methods

During two years, from each season, dairy products and raw cow milk were purchased from three street markets of Mexico City and three cow stables of the state of Mexico. 120 samples were collected and analysed for the presence of several food-borne pathogens, including mycobacteria. Mycobacteria speciation was done using three molecular markers: *hsp*65, *rrs*, and *rpo*B genes. NTM isolates were further characterized by: biofilm and motility assays, and antimicrobial susceptibility testing. **Results.** A total of 72 cheese, 24 cream, and 24 raw milk samples were collected. From 3 cheese, 5 cream, 3 raw milk samples (n=11, 9.1%), mycobacteria strains were isolated. Only one NTM species was recovered from 8 samples: *M. fortuitum* (n=3), *M. porcinum* (n=3), *M. rhodesiae* (n=1), *M. abscessus* (n=1); while *M. abscessus* and *M. chelonae*, were concurrently recovered from three samples. Biofilm formation and sliding motility were observed among 93% and 71% of NTM isolates, respectively. Besides 57% of the isolates were resistant to Linezolid and 50% to Clarithromycin.

Conclusions

Consumption of cheese, cream and raw milk harbouring NTM with virulence properties may represent a public health risk.

FEMS7-3320
Food Microbiology - Part III

HUMAN BIOTOPE HEALTHY STATUS SUPPORTED BY GLYCOCONJUGATES RECOGNITION PROBIOTIC SYSTEMS

M. Lakhtin[1], V. Lakhtin[1], S. Afanasiev[1], A. Bajrakova[1], V. Aleshkin[1]
[1]Medical Biotechnology, G.N.Gabrichevsky Institute of Epidemiology and Microbiology, Moscow, Russia

Background
Microbial communities participate in biotope healthy status.
Objectives
One more strategy to support healthy biotope.
Methods
Glycoconjugates recognition probiotic systems **(**GRPS) of probiotic bacterial cultures; intestinal and urogenital microbial clinical strains were used. Microbes were growth in standard media and conditions in the absence or presence of GRPS.
Conclusions
1. *Features of GRPS*. GRPS are localized, organized and act as cell layer mosaic surface and secreted truncated/ modified and assembled active or activated systems which imitate probiotics. GRPS are represented by synergistically cofunctioned bifidobacteria and lactobacilli probiotic lectins and their complexes, glycosyl hydrolases. GRPS function as soluble signals and cell pools. GRPS reveal properties of quorum sensing molecules, cross-talking agents, metabolomebiotics, net switchers; antifungal and antistaphylococcal effectors. **2.** *Biotope communications. L.acidophilus, L.casei* and *L.brevis* suppress or significantly limit fungal growth of *C.albicans, C.tropicalis, C.krusei* and/or *C.albicans+A.niger*; influence subspecies microecological niches within fungal group (*C.albicans* and *C.tropicalis*). *L.acidophilus* and *L.casei* probiotic-like pools (main sources of GRPS) contain leader strains which desorganize communications between *Candida* species/ subspecies or within *C.albicans* strain massive. GRPS help in elimination of changeable lactobacillus strains. Biotope *Candida* pools contain leader strains which desorganize *Lactobacillus* species communications. **3.** *Strategy to support healthy biotope*: screening probiotic and potentially pathogenic yeast-like leader strains; delivery of synbiotic (probiotic lectins and strains); elimination of antimycotic sensitive leader strains. **4.** Universal conception of healthy biotope microbiocenose involving GRPS and leader strains is proposed.

FEMS7-2843
Food Microbiology - Part III

STRESS RESISTANCE OF BIOFILM AND PLANKTONIC CELLS OF LACTOBACILLUS SPP

M. Olszewska[1], A. Kocot[1], A. Nynca[2]
[1]University of Warmia and Mazury in Olsztyn,
Faculty of Food Science- Chair of Industrial and Food Microbiology, Olsztyn, Poland
[2]University of Warmia and Mazury in Olsztyn,
Faculty of Biology and Biotechnology- Laboratory of Molecular Diagnostics, Olsztyn, Poland

Backgrounds

Although the biofilm formation by *Lactobacillus* species has been reported, stress responses of Lactobacilli biofilms associated with food production and spoilage have not been the subject of detailed studies. As a result, we have elucidated in detail the difference in resistance between planktonic and biofilm Lactobacilli cells to food acidulants, preservatives, disinfectants.

Objectives

1. Assess the ability to form biofilms by *Lactobacillus* species that cause food contamination or deterioration. 2. Examine the response of *Lactobacillus* biofilm formers to acetic acid, sucrose, ethanol, sodium dichloroisocyanurate-based and didecyl dimethyl ammonium chloride-based biocides. 3. Gain a better understanding of single cell physiology and properties of planktonic versus biofilm cells.

Methods

The biofilm formation was tested using the microtiter plate assay. Before survival test, biofilm formers were grown either as planktonic cultures or biofilms on polycarbonate coupons. For viability studies, we applied flow cytometry (FCM) which provided the insight into damage of cell membrane integrity. Plate counts were performed for cell culturability and/or cell recovery evaluation.

Conclusions

Isolates showed differences in the ability to form biofilms and tolerance to stress conditions relevant for food processing. Noteworthy is that biofilm cells were more resistant to ethanol, which strongly inhibits the growth of bacteria and is important in food preservation, and the treated biofilm cells were injured or damaged to a lesser extent than planktonic counterparts. These results indicate the significance of controlling Lactobacilli biofilms in the food industry.

This study was supported by a grant from the National Science Centre, Poland, no. 2014/13 /D/NZ9/04803.

FEMS7-1279
Food Microbiology - Part III

CORRELATION BETWEEN BIOFILM FORMATION AND COMPOSITION AND MOLECULAR ASPECTS OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

E.A. Oniciuc[1,2], F.I. BUCUR[1], D. RODRÍGUEZ-LÁZARO[2], V. BARBU[1], M. Hernández[3,4], A.I. NICOLAU[1]
[1]Dunarea de Jos University of Galati, SAIABA Department, Galati, Romania
[2]University of Burgos, Department of Biotechnology and Food Science, Burgos, Spain
[3]Instituto Tecnológico Agrario de Castilla y León, Laboratory of Molecular Biology and Microbiology, Valladolid, Spain
[4]Universidad de Valladolid, Departamento de Ingeniería Agrícola y Forestal, Palencia, Spain

Backgrounds

There is a growing concern regarding potential transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) by illegal entrance of food to Europe. From a food safety perspective, different MRSA lineages can be acquired *via* food manipulation and/or consumption, and consequently circulation of multidrug resistant strains may arise. Resistance to antimicrobial agents can be also related to different responses in attachment and biofilm formation.

Objectives

More information regarding MRSA strains capacity to build biofilms and their composition of a matrix of extracellular polymeric substances involved in the attachment and colonization of food-contact surfaces is presented.

Methods

Forty-nine MRSA isolates recovered from animal food origin were evaluated for the ability of biofilm formation (24h) using TSB supplemented with 0.4% glucose. Experiments were performed in static conditions on 96-well hydrophobic surfaces at 37°C and a starting inoculum of ~ 10^6 CFU/mL. Biofilm compositions (48h) was evidenced by confocal laser scanning microscopy (CLSM) after exposure to three types of dyes: SYTO9 (nucleic acids), SYPRO Ruby (proteins), and WGA-TRITC (*N*-acetyl-D-glucosamine residues). Image stacks of biofilms were analysed further.

Conclusions

All MRSA isolates tested have the capacity to form biofilms. Time prolongation (48h) shows strong biofilm formation (OD>3) for 8 isolates, while the remaining 41 strains were weakly or moderate biofilm formers. A correlation between the formation of biofilms and MRSA lineages was observed, whereas a higher biomass was observed for those harboring SCC*mec* type IV. Half of the isolates formed flat and compact structures, while the rest of them developed highly fluorescent cell aggregates areas within the three-dimensional structures.

FEMS7-0725
Food Microbiology - Part III

CHARACTERIZATION OF BACTERIOPHAGE–RESISTANCE IN ORAL PHAGE THERAPY

J. Otero[1], M. Sánchez-Osuna[1], P. Cortés[1], M. Llagostera[1]
[1]Universitat Autònoma de Barcelona,
Grup de Microbiologia Molecular. Departament de Genètica i de Microbiologia. Facultat de Biociència s. Edifici C, 08193 Cerdanyola del Vallès, Spain

Backgrounds

Bacteriophages have proved to be interesting therapeutics agents. Nevertheless, bacteriophage therapy may result in the selection of resistant bacterial cells, preventing an effective treatment.

Objectives

To study the emergence of bacteriophage-resistant *Salmonella* during *in vivo* oral phage therapy, with a cocktail composed by three virulent bacteriophages, and also in *in vitro* infected cultures.

Methods

The *in vivo* emergence of cells resistant to bacteriophages was studied in commercial broilers infected with *Salmonella enterica* serovar Typhimurium ATCC14028 after oral therapy with a cocktail of three bacteriophages. The selection of resistant cells *in vitro* was studied by infecting liquid cultures of ATCC14028 with the three bacteriophages individually or as a cocktail.

Conclusions

Resistance was not detected in the treated broilers after oral phage therapy, while resistant cells were found in the untreated ones. These results can be explained by the significant reduction of the *Salmonella* population due to the bacteriophage treatment and by the lower fitness and persistence of resistant cells in the cecum. Moreover, *Salmonella* isolates with an atypical behaviour to bacteriophages were recovered from untreated and treated animals.
Unlike the results found *in vivo*, different populations of resistant cells were found in the *in vitro* bacteriophage infected cultures. Results seem indicate that the emergence of bacteriophage resistance could be different in *in vivo* and *in vitro* scenarios, which suggest the need for caution in extrapolating *in vitro* results. Furthermore, a deeper phenotypic and genomic characterization of the different resistance cells is required in order to understand the mechanisms of resistance to bacteriophages.

FEMS7-0967
Food Microbiology - Part III

A COMPARATIVE STUDY ON PULLULAN PRODUCTION BY VARIOUS AUREOBASIDIUM PULLULANS STRAINS

G.N. Kara[1], M.T. Bozdemir[2], Z.Y. Ozbas[3]
[1]Hitit University, Food Engineering, Corum, Turkey
[2]Hacettepe University, Chemical Engineering, Ankara, Turkey
[3]Hacettepe University, Food Engineering, Ankara, Turkey

Backgrounds

Aureobasidum pullulans is a yeast-like fungus that is particularly known as a producer of an extracellular polysaccharide; pullulan. Pullulan is an economically important polymer which is used in food, pharmaceutical, agricultural and chemical industries because it is nontoxic, biodegrable, tasteless, odorless, white and non-hygroscopic.

Objectives

The aim of this study was to investigate pullulan production characteristics of seven *A. pullulans* strains in a batch system.

Methods

A. pullulans strains BJ20p4, TreCisz2, G. Kaukaska B-1, CCF 4532, MAFF 425047, 100716 and AZ-6 were used in the experiments for pullulan production. The shake-flask fermentations were performed in 150 mL liquid media containing (g/L) sucrose; 50, $(NH_4)_2SO_4$; 2.0, yeast extract; 3.0, K_2HPO_4; 5.0, $MgSO_4.7H_2O$; 0.2 and NaCl; 1.0, at 28ºC in a waterbath shaker with a shaking rate of 100 strokes/min. Samples from the cultures were taken at certain intervals and centrifuged at 5000 rpm for 20 minutes. Pullulan was precipitated from the supernatant using two volume of cold ethanol per volume of supernatant. The pullulan content of the precipitate was determined by enzymatic hydrolysis to maltotriose with pullulanase followed by determination of reducing sugar equivalents by using the dinitrosalisylic acid method. Obtained pullulan was characterized by FT-IR spectroscopy.

Conclusions

Among the examined isolates, a domestic pinkish strain; *A. pullulans* AZ-6 was found the best candidate for pullulan production. The maximum exopolysaccharide and pullulan concentrations were obtained as 17,60 and 17.56 g/L, respectively by using this strain. The FT-IR spectra of the exopolysaccharide produced by *A. pullulans* AZ-6 and of the standard sample were almost identical.

FEMS7-0832
Food Microbiology - Part III

MULTIFUNCTIONAL EFFECTS OF VIABLE AND HEAT-KILLED LACTOBACILLUS PLANTARUM LN1 ISOLATED FROM KIMCHI

H.J. Jang[1], M.W. Song[1], J.H. Lee[1], H.D. Paik[1]
[1]Konkuk university, Department of Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea

Backgrounds

Recently, many investigators have studied heat-killed lactic acid bacteria (LAB) because heat-killed LAB are stored more effectively than viable LAB for long periods and are transported easily. Nonetheless, some researchers announced that heat-killed LAB are not probiotics regardless of functionality.

Objectives

We compared multifunctional effects of viable LAB and heat-killed LAB to determine whether these effects are as good in heat-killed LAB as viable LAB and to increase the use of the probiotics in industries via this study.

Methods

Lactobacillus plantarum Ln1 was incubated at 37°C for 15 h. Heat-killed LAB were prepared by heat-treatment at 80°C for 30 min. They were centrifuged, and the pellet was washed with 0.1% peptone water and resuspended. The antioxidant, anti-inflammatory, and anti-cancer effects of viable and heat-killed LAB were evaluated by a β-carotene bleaching assay, NO production in LPS-stimulated RAW 264.7 cells, and an MTT assay.

Conclusions

The antioxidant effect was better in heat-killed LAB 49.97% and 58.33% for viable and heat-killed LAB, respectively. NO production was reduced by treatment with *L. plantarum* Ln1 at 28.19 μmol compared to the control LPS-stimulated cells and untreated cells. MTT results of viable LAB were 68.63%, 89.05%, and 83.05%, and those of heat-killed LAB were 67.57%, 32.31%, and 20.85%, in HeLa, AGS, and HT-29 cells, respectively. Although MTT results are better for viable cells than heat-killed LAB, heat-killed LAB seem to have an anti-cancer effects. Thus, our findings about heat-killed LAB may provide useful information to the probiotics industry.

FEMS7-0854
Food Microbiology - Part III

PROBIOTIC PROPERTIES OF SACCHAROMYCES CEREVISIAE ISOLATED FROM TRADITIONAL FERMENTED FOODS WITH A CHOLESTEROL-LOWERING EFFECT

J.Y. Hong[1], S.H. Son[1], S.H. Yi[2], N.K. Lee[1], S.P. Hong[2], J.M. Park[3], H.D. Paik[1]
[1]Konkuk university, Department of Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea
[2]Korea Food Research Institute, Department of Strategic Food Research, Seongnam, Republic of Korea
[3]Korean Culture Center of Microorganisms, Korean Culture Center of Microorganisms, Seoul, Republic of Korea

Backgrounds

Probiotics are defined as live micro-organisms that confer a health benefit on the host. Probiotic yeast *Saccharomyces cerevisiae* has been extensively studied in terms of its ability to limit inflammation and infection in the gastrointestinal tract. In recent studies, the ability of probiotic strains to hydrolyze bile salts has often been included among the criteria for probiotic-strain selection. Bile salts play an important role in the digestion of lipids in vertebrates and are synthesized and conjugated to either glycine or taurine in the liver.

Objectives

The present work was conducted to obtain information about the probiotic properties and functional materials of 3 strains of *S. cerevisiae* (KCCM 200280, 200281, and 200284) isolated from Korean pickled cucumbers.

Methods

Strains (KCCM 200280, 200281, and 200284) showed a good survival rate in artificial gastric acid and bile acid (>89%). In addition, enzymatic activity was assessed using the API kit, and beneficial enzymatic activities were detected. The *S. cerevisiae* strains showed strong attachment, 8.3%, 20.9%, and 16.4%, respectively, to intestinal HT-29 cells. Deconjugation of bile salts and precipitation of soluble cholesterol by *S. cerevisiae* in the bile salt-YM medium were measured. In the medium containing taurocholate (TCA) and glycocholate (GCA), all *S. cerevisiae* strains showed strong resistance to bile salts (>87%).

Conclusions

Therefore, these results suggest that *Saccharomyces cerevisiae* is applicable as a probiotic having a cholesterol-lowering effect.

FEMS7-1050
Food Microbiology - Part III

THE EFFECT OF FERMENTED PARSLEY ROOTS JUICE VS. SODIUM NITRITE ON MINCED PORK SHELF-LIFE

C. Papuc[1], G. Stefan[1], V. Nicorescu[1], C. Predescu[1], I. Nicorescu[2], C. Petrescu[3]
[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Veterinary Medicine, Bucharest, Romania
[2]Institute of Hygiene and Veterinary Public Health, Food Microbiology, Bucharest, Romania
[3]Scientica Foundation, Scientica, Bucharest, Romania

Backgrounds

In response to consumers' demand for natural preservatives, vegetable juices rich in natural nitrites may be an alternative to sodium nitrite.

Objectives

The objective of this study was to evaluate the effects of natural nitrites from parsley (*Petroselinum crispum*) roots fermented juice (NPFJ) on minced pork (MP) subject to refrigeration, comparatively to sodium nitrite (SN).

Methods

MP was treated with NPFJ in relation of 0, 50, 100, 150 ppm nitrite, and with SN in the same relations. Treated MP was refrigerated at 4ºC for 12 days. Microbiological parameters (total viable count (TVC), coliform bacteria (CB), *Enterobacteriaceae*, and *E coli*) and physico-chemical parameters (pH, volatile basic nitrogen (VBN) and thiobarbituric acid reactive substances (TBARS)) were determined every two days. NPFJ had similar effects on MP shelf-life, comparatively with SN at the same concentration. The best results were found in MP treated with NPFJ 150 ppm; the microbial counts varied in days 0-12 in the relations: TVC 5.4-5.6 log cfu/g, CB 2.1-3.2 log cfu/g, *Enterobacteriaceae* 2.1-2.7 log cfu/g, and *E. coli* <1 log cfu/g. For the physico-chemical parameters, the variations found were in the relations: pH 5.85-6.39, VBN 17.83-24.69 mg N/100 g, and TBARS 0.25-0.47 mg MDA/kg.

Conclusions

Natural nitrites from parsley roots fermented juice are highly effective in preserving minced pork and show potential to be used as preservatives in meat industry. This work was carried out through *Partnerships in priority areas* Program – PN II, implemented with the support of MEN – UEFISCDI (Romania), project nr. 149/2014.

FEMS7-1838
Food Microbiology - Part III

INFLUENCE OF OSMOTIC PRESSURE ON THE GROWTH OF S. CEREVISIAE IN THE MERLOT VARIETY

I. Pardo[1], L. Polo[1], L. Andrés[1], C. Berbegal[1], S. Ferrer[1]
[1]ERI BioTecMed, ENOLAB, Burjassot, Spain

Backgrounds

The climate change effect leads to a rapid growth of the grapevine and an imbalance in grape maturation. In a normal ripening period, sugars accumulate at an adequate level, maintaining the acidic structure and resulting in a balanced wine. An excessive concentration of sugar can delay the start or arrest alcoholic fermentation, due to the high osmotic pressure. Therefore, yeasts are not able to perform the fermentation because the high concentrations of sugar slow the osmotic processes of cell membranes and may decrease their growth.

Objectives

Studying the behaviour of 14 native *Saccharomyces cerevisiae* strains - previously isolated from ecological Merlot industrial vinifications - versus different concentrations of sugar.

Methods

A natural industrial Merlot grape must was added with glucose and fructose (50%/50%) to reach 22, 24 and 25° Brix. Growth kinetics, glucose/fructose consumption, and ethanol, glycerol and acetic acid production along alcoholic fermentation were monitored.

Conclusions

An increase of sugar content reduces the maximum biomass, the growth rate of yeasts, or both parameters. Moreover, the higher initial sugar concentration is (glucose and fructose), the higher ethanol, glycerol and acetic acid concentrations are produced. These increases, nevertheless, are not proportional to the amount of initial sugar. The most sensitive strains to high sugar concentrations are 2e and 2g, since they yield the lowest values of maximum biomass, growth rates or both, and the most resistant strains are 7a, 7d, 7e and 10b.

FEMS7-0766
Food Microbiology - Part III

THE CHANGE OF MICROBIAL FLORA BY STARCH GRANULES AND ISOLATION OF RAW STARCH-DEGRADING MICROORGANISMS IN BOVINE RUMEN FLUID

C.S. Park[1], D.H. Jung[1], Y.J. Eeom[1], G.Y. Kim[1]
[1]Kyung Hee University, Food Science and Biotechnology, Yongin, Republic of Korea

Backgrounds

Granular starches are found in a number of plants and the most common carbohydrate in human and animal diets. In general, they are incompletely digested due to their size and molecular conformation.

Objectives

The objectives in this study is to observe the change of microbial flora in rumen fluid of *Bos taurus coreanae* by starch granules and to isolate starch granule-degrading microorganisms.

Methods

The change of microbial flora in rumen fluid of Korea bovine, *Bos taurus coreanae* was analyzed by metagenomics analysis using starch granules composed of 70% amylopectin and 30% amylose. From the starch granule-containing media, several strong starch granule-degrading microorganisms were isolated and identified in anaerobic growth condition. The utilization profile of various resistance starches by isolated *Bifidobacterium choerinum* was compared with the human intestinal amylolytic anaerobes.

Conclusions

Even though the major microbial strain in rumen fluid was *Succiniclasticum* sp., *Streptococcus* sp. was firstly thrived followed by *Lactobacillus* sp. in starch granule-containing media. Besides, the presence of *Bifidobacterium* sp. was continuously observed. Several strong starch granule-degrading microorganisms were identified and isolated. These strains were *Bifidobacterium pseudolongum* and *B. choerinum.* Particularly, both microorganisms were strongly attached to starch granule, and also exhibited resistance starch-hydrolyzing activities. In fact, *B. choerinum* showed a fast hydrolyzing ability towards different type of raw and resistant starches by degrading 80% of substrates within 5 hours. These results suggest that *Bifidobacterium* sp. is one of the main granular starch degrader in rumen microbial flora and could be further studied as a probiotic candidate.

FEMS7-0649
Food Microbiology - Part III

PROFILE AND CHARACTERISTICS OF LACTIC ACID BACTERIA AND BACTERIOPHAGE DURING KIMCHI FERMENTATION

J.H. Park[1], Y.D. Lee[2], W.J. Park[1], G.Y. Lim[1]
[1]Gachon University, Department of Food Science and Biotechnology, Seongnam, Republic of Korea
[2]Seowon University, Department of Food Science and Engineering, Cheongju, Republic of Korea

Backgrounds

Many foods have been fermented based on lactic acid bacteria.

Objectives

Objective of the present study was to investigate lactic acid bacteria (LAB) and bacteriophage of succession.

Methods

The fermentation on kimchi was divided into 7℃ and 20℃. As fermentation started at 7℃ and 20℃, the proportion of LAB increased and amount of virus-like particle (VLP) changed.

Conclusions

According to the fermentation temperature, the quantitative change of bacteriophage showed at different time. As a result of microbial analysis using pyrosequencing at the change of VLP, change of the dominant LAB in each period was shown. *Leuconostoc* was the dominant species in the initial and middle stage of fermentation, and *Lactobacillus* in the late stage of fermentation at 7℃. *Weissella* did not show after the initial stage of fermentation at 7℃. *Lactobacillus* was the dominant species on the 2 day fermentation at 20℃. Total 17 Bacteriophages were isolated from 7℃ fermentation. Bacteriophages showed changes with the succession of LAB. In morphological analysis, *Weissella cibaria* bacteriophages were belonged to *Podoviridae* family. *Leuconostoc lactis* and *Leuconostoc citreum* bacteriophages were belonged to *Myoviriade* family. Selected 6 strain bacteriophages were stable in the acidic condition. Especially, φ1D.4.1 decreased only by 5.7 log PFU/ml at pH 2 for 30 minutes. Therefore, bacteriophage with high acid tolerance might be involved in the succession of LAB during natural fermentation of kimchi.

FEMS7-0650
Food Microbiology - Part III

CHARACTERIZATION AND GENOME SEQUENCE ANALYSIS OF BECP4 PHAGE FOR SHIGA TOXIN PRODUCING E. COLI

J.H. Park[1], Y.D. Lee[2], M. Kim[2], S.C. Cho[2], G.Y. Lim[1]
[1]Gachon University, Department of Food Science and Biotechnology, Seongnam, Republic of Korea
[2]Seowon University, Deaprtment of Food Science and Engineering, Cheongju, Republic of Korea

Backgrounds

Shiga toxin producing *E. coli* (STEC) have also been reported to contaminate fresh produce, leading to a high risk of infection in vulnerable populations such as children and the elderly. Recently, phages have been extensively studied and have been used for a variety of practical applications such as for biocontrol of STEC.

Objectives

In this study, BECP4 phage was isolated from bovine fecal samples, and analyzed for various characteristics and genome sequence.

Methods

BECP4 phage was analyzed for genome information and characterized biochemically.

Conclusions

Morphological analysis showed that BECP4 phage belonged to the family *Myoviridae*. The genomic sequence of BECP4 phage was comprised of circular double-stranded DNA and was 138,462 bp, with a G+C content of 43.65%. The genome showed 212 putative ORFs, and the 6 tRNAs were identified. In stability, BECP4 phage was susceptible to temperatures above 70℃. However, its stability was reduced to 3-4 log PFU/ml after 60 min in 70% ethanol. The host spectrum using 34 STEC strains demonstrated that the BECP4 phage was able to lyse 19 STEC strains.
Thus, *Myoviridae* BECP4 phage with high stability might serve as effective biocontrol agents to simultaneously reduce STEC in food and remove STEC from biofilms.

FEMS7-0206
Food Microbiology - Part III

TEMPERATURE PRECONDITIONING AFFECTS MICROBIAL COMMUNITY DYNAMICS OF POSTHARVEST CHERRY TOMATOES (LYCOPERSICUM ESCULENTUM VAR. CERASIFORME) DURING REFRIGERATION STORAGE

K.M. Park[1], M.C. Jeong[2], M. Koo[1,2]
[1]Korea University of Science and Technology, Food Biotechnology, Daejon, Republic of Korea
[2]Korea Food Research Institute, Food Storage and Distribution, Gyeonggi-do, Republic of Korea

Backgrounds

Microbial community structure of fruit and vegetable are strongly influenced by environmental conditions such as temperature. Many research focused on the microbiological and sensory quality changes and overall diversity and composition of bacterial communities by environmental conditions are poorly understood.

Objectives

The objectives were (1) to investigate microbial community variation by temperature preconditioning and (2) to determine the relationship between spoilage bacteria development and temperature preconditioning during storage.

Methods

After harvest, fruit temperature(FT) by preconditioning were controlled in 4 group: FT-5(immediately stored at 5℃); FT-10(10℃); FT-20(20℃); and FT-30(30℃) , after that, stored at 5 ℃. Microbial community analysis was performed by MALDI-TOF MS and VITEX 2 compact.

Conclusions

The communities on each preconditioned fruit were significantly distinct. After preconditioning treatment, bacterial composition consisted of 19 different genera and 52% of total bacterial isolates belonged to *Staphylococcus xylosus*, 14% to *Bacillus pumilus* and 8% to *Pantoea agglomerans*. The most abundant strains of FT-5 and FT-10 identified as *Bacillus pumilus*. In contrast, *Staphylococcus xylosus* were found with abundance above 60% in FT-20 and FT-30. At the end of refrigeration storage, bacterial diversity dramatically decreased to 9 genera and most strains belonged to vegetable spoilage bacteria. *Pseudomonas fluorescens* on FT-5 and FT-10 showed high abundance and *Rahnella aquatilis* was dominated in FT-20 and FT-30. We found that temperature preconditioning after harvest had a considerable impact on the microbial community. These observations will provide new sight to enhance microbiological safety of cherry tomato or to prolong postharvest shelf life based on microbial community-environmental condition relationships.

FEMS7-1280
Food Microbiology - Part III

PRESERVATION OF A SMEAR SURFACE-RIPENED CHEESE ECOSYSTEM BY FREEZING AND FREEZE-DRYING: A PROOF OF CONCEPT

W. Cao[1], F. Irlinger[1], T. Cattenoz[1], M.N. Leclercq-Perlat[1], S. Passot[1], F. Fonseca[1]
[1]UMR GMPA- AgroParisTech- INRA, Université Paris-Saclay, Thiverval-Grignon, France

Backgrounds

The preservation of microbial resources of interest for cheese making and obtained from traditional technological uses (whey, indigenous microbial flora, wooden Gerles), appears as an important issue to safeguard the overall biodiversity of microbial cheese communities. Many protocols have been developed for long-term and stable storage of axenic cultures, while few preservation methodologies have been described for microbial consortia.

Objectives

The objective of this work was to develop preservation methods to stabilize entire microbial cheese communities without multiplication or isolation.

Methods

A simplified microbial community, composed of 9 micro-organisms and capable of reproducing the complex metabolic pattern of cheese maturation was used. Three independent cheese productions were performed. Stabilization conditions were applied, including formulation and different operating conditions of freezing, freeze-drying and storage. The survival of each micro-organism of the cheese consortia was quantified by microbiological counts in selective media at days 1 and 21 of the ripening and after freezing, freeze-drying and storage of cheese samples. Physical characterization was performed by measuring water activity and water content of stabilized samples. In order to better understand the mechanisms underlying preservation, thermal events such as the glass transition temperatures of cheeses were identified by differential scanning calorimetry.

Conclusions

This work made it possible a better knowledge of the resistance to stabilization processes of each micro-organism within the consortium. Moreover, a cheese production was successfully carried out by using the best stabilized cheeses for inoculation, showing that it is possible to preserve and then re-use the whole of a microbial community of interest.

FEMS7-2821
Food Microbiology - Part III

INHIBITION OF PENICILLIUM BY LACTOBACILLUS SPP. ISOLATED FROM MILK.

J. Pereira[1], E. Antonio Rios[1], J. Angel Santos Buelga[1], G.L. María Luisa[1], L.D. Teresa María[1]
[1]Universidad de León, 1Department of Food Hygiene and Food Technology- Veterinary, León, Spain

Backgrounds

In order to control spoilage and toxigenic fungi in cheese, chemical preservatives are commonly used. Within the biocontrol techniques, which are highly valued today, lactic acid bacteria (LAB) are being investigated. In an earlier study by the authors, 93 strains of LAB with antagonistic capacity against *Penicillium commune* (M35, CECT 20940) and *Penicillium nordicum* (M32, CECT 20939) (strains of cheese origin) were isolated. After confirming the antagonistic capacity, 57 strains were selected, 34 showed the greatest capacity (with inhibition halos of 8-12 mm). In another study by the authors, 5 strains were identified, where 2 represented *Lactobacillus casei*, and the rest were *L. parabuchneri*, *L. rhamnosus* and *L. plantarum*.

Objectives

The aim of this study is to identify the 29 remaining strains and analyze their activity against another 5 strains of *Penicillium* (4 *Penicillium commune* and 1 *Penicillium verrucosum*).

Methods

The identification was carried out with a sequencing of DNA 16 S technique. The studies on *Penicillium* inhibition were carried out with a "spot on the lawn" plate technique.

Conclusions

Up to now, it was possible to identify 23 of the 29 strains. The species identified were: *Lactobacillus paracasei* (32.1%), *Lactobacillus casei* (25%), *Lactobacillus plantarum* (17.9%), *Lactobacillus parabuchneri* (7,1%), *Lactobacillus brevis* (3.6%), *Lactobacillus zeae* (3.6%) and *Lactobacillus fermentum* (3.6%). According to the antifungal capacity results against *Penicillium* strains, *Lactobacillus casei* was the species that revealed greater inhibition capacity.

FEMS7-1158
Food Microbiology - Part III

NATURAL ANTIMICROBIALS AND HIGH PRESSURE TREATMENTS ON THE INACTIVATION OF LISTERIA MONOCYTOGENES AND THE CHARACTERISTICS OF SLICED DRY-CURED HAM

A. Pérez-Baltar[1], A. Serrano[1], R. Montiel[1], D. Bravo[1], M. Medina[1]
[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Food Technology, Madrid, Spain

Backgrounds

Listeria monocytogenes is a bacterial pathogen able to grow under different stress conditions (refrigeration temperatures, low pH or high levels of NaCl) and commonly found in the food industries, including tools and equipment. Combined inactivation treatments provide greater protection against this pathogen, improving the safety of ready-to-eat (RTE) meat products.

Objectives

The effect of high pressure processing (HPP) combined with enterocin and thymol on *L. monocytogenes*, total viable counts and the physicochemical, rheological and colour characteristics of sliced dry-cured ham was investigated.

Methods

A four-strain cocktail of *L. monocytogenes* was used to inoculate sliced dry-cured ham at 10^6 cfu/g. Enterocin extract and thymol were added to dry-cured ham slices at 1054 AU/g and 1.25 mg/g, respectively. Samples were pressurized at 450 MPa for 10 min and stored at 4 and 12 °C during 30 d. *L. monocytogenes* and total viable counts were performed at 1, 7, 15 and 30 d. Likewise, pH, water activity (a_w), colour and texture were determined at 1, 15 and 30 d in non-inoculated samples.

Conclusions

Enterocin exhibited a strong antimicrobial effect against *L. monocytogenes* in dry-cured ham, whereas a regrowth was registered from day 15 onwards. 450 MPa or thymol showed low activity against the pathogen. Combined treatment of enterocin and HPP exhibited a synergistic bactericidal effect during 30 d at 4 and 12 ºC, and could be a useful technology to avoid the growth of *L. monocytogenes* in case of contamination during the processing. Concerning physicochemical, rheological and colour characteristics, slight modifications were detected throughout the storage.

FEMS7-1170
Food Microbiology - Part III

HIGH PRESSURE TREATMENTS ON THE INACTIVATION OF LISTERIA MONOCYTOGENES AND EFFECT ON THE PHYSICOCHEMICAL CHARACTERISTICS OF DEBONED DRY-CURED HAM

R. Montiel[1], A. Pérez-Baltar[1], A. Serrano[1], M. Medina[1]
[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Food Technology, Madrid, Spain

Backgrounds

Dry-cured ham can be contaminated with *Listeria monocytogenes* during the industrial processing, especially during deboning, slicing, packaging and other handling processes, which has become in a great concern for the manufacturers. Therefore, additional hurdles are needed to reduce or eliminate the presence of such pathogen in this processed ready-to-eat (RTE) meat product.

Objectives

The aim of this work was to investigate the effect of high pressure processing (HPP) on *L. monocytogenes* and the physicochemical characteristics of deboned dry-cured ham.

Methods

A four-strain cocktail of *L. monocytogenes* was inoculated in the external and internal side of deboned dry-cured hams at 10^{5-6} cfu. Hams were vacuum-packaged and pressurized at 450 and 600 MPa for 10 and 5 min, respectively, and stored under strict refrigeration temperatures (4 °C) and temperature abuse conditions (12 °C) during 60 d. Inoculated areas were taken from each ham at 1, 30 and 60 d to perform *L. monocytogenes* counts. Non-inoculated samples were taken at each sampling time for the determination of pH, water activity (a_w), moisture content, sodium chloride and nitrites content.

Conclusions

Pressurization at 600 MPa significantly reduced *L. monocytogenes* population in the internal side of dry-cured ham. The low water content in the external side protected the pathogen against HPP immediately after treatments, although *L. monocytogenes* counts significantly diminished during 60 d of storage at 4 and 12 ºC compared to non-treated samples. Regarding physicochemical characteristics, slight modifications in pH, a_w, moisture content, sodium chloride and nitrites content were recorded throughout the storage.

FEMS7-2852
Food Microbiology - Part III

BIOGENIC AMINE PRODUCTION IN A COLLECTION OF LACTIC ACID BACTERIA ISOLATED FROM GOAT MILK

A. Picon[1], P. Gaya[1], C. Escudero[1], C. Sánchez[1], M. Nuñez[1]
[1]INIA, Food Technology, Madrid, Spain

Backgrounds

Biogenic amines (BA) are low molecular weight organic bases that may cause food poisoning episodes. In fermented foods, the availability of substrates and the appropriate conditions for bacterial growth and decarboxylase activities cause their biosynthesis and accumulation. The most important BA in fermented foods are tyramine, histamine, putrescine and cadaverine, respectively produced from tyrosine, histidine, ornithine and lysine.

Objectives

To investigate the production of BA in a collection of 298 lactic acid bacteria isolated from goat milk.

Methods

BA production was evaluated in the decarboxylase agar medium (Bover-Cid and Holzapfel, 1999) after incubation at 30-37°C for 4 days. From isolates showing darkening or change in colour, total genomic DNA was extracted and PCR amplification with specific PCR primers for decarboxylase structural genes was performed (De las Rivas et al., 2005). Confirmation of BA production was performed by HPLC.

Conclusions

Darkening or change in colour in the decarboxylase agar medium with one or several precursor amino acids was recorded for 46 *Enterococcus*, 12 *Lactobacillus*, 10 *Lactococcus*, 5 *Leuconostoc*, 2 *Carnobacterium* and 2 *Weissella* isolates. Only the tyrosine decarboxylase gene could be amplified, from 46 *Enterococcus* isolates, the two *Carnobacterium* isolates and one *Lactobacillus* isolate. Tyramine production was confirmed by HPLC for all those 49 isolates.

FEMS7-2819
Food Microbiology - Part III

CHARACTERIZATION AND OPTIMIZATION OF TECHNOLOGICAL PROCESSES FOR THE PREPARATION OF ASTURIAN SAUSAGE THROUGH THE USE OF METAGENOMICS

N. Prado[1], M. Sampayo[1], P. González[1], J. Díaz[1]
[1]ASINCAR, Laboratorio e I+D+i, Noreña, Spain

Backgrounds

The manufacture of Asturian Sausage requires a fermentative process followed by a stage of maturation to attain its typical sensorial properties. Traditionally, microorganisms present in the meat or as well as those coming from activities like manipulation or industrial environment, are responsible of the fermentative processes. The problem is that not all the microorganisms present in the raw materials contribute to a good fermentation processes and sometimes they are responsible of product spoilage.

Objectives

Main goal is the use of sequencing techniques, Metagenomics, with the aim of improving the elaboration process of Asturian Sausage.

Methods

Metagenomics is a new scientific field that allows the analysis of a large number of nucleic acid fragments from a sample as well as the identification of which microbial species are present and in which relative percentage. This technique has advantages in comparison to conventional microbiological analysis techniques since it allows the identification of any microorganisms, including non-cultivable ones, and the determination of the phylogenetic relationships between them.Using Metagenomics we will be able to analyze the microorganisms responsible of the technological processes that take place during the elaboration of the fermented raw-cured sausage.

Conclusions

The determination of microbial ecology through the different production stages of the Asturian Sausage has allowed to obtain complete information about the fermentation process. This will allow the design of specific strategies to improve food safety as well as the development of new product processes.

FEMS7-1098
Food Microbiology - Part III

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF DIFFERENT BLACK GRAPE POMACE EXTRACTS RICH IN POLYPHENOLS

C. Predescu[1], I. Nicorescu[2], G. Stefan[1], V. Nicorescu[1], A.E. Rus[3], C. Petcu[1], C. Papuc[1]
[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, Bucharest, Romania
[2]Institute of Hygiene and Veterinary Public Health, Food Microbiology, Bucharest, Romania
[3]Banat's University of Agricultural Sciences and Veterinary Medicine of Timisoara, Food Science, Timisoara, Romania

Backgrounds

The pomace which results after obtaining the wine can be used as a source of antioxidants such as polyphenols.

Objectives

The aim of the study was to determine the antioxidant activity (AOxA) and antimicrobial activity (AMA) of polyphenols from black "Feteasca neagra" grape pomace (BGP) extracted in ethanol, methanol, acetone, chloroform, and water at room temperature for 12 hours.

Methods

Total polyphenolic content (TPC) was determined with Folin-Ciocalteu reagent and expressed as mg gallic acid equivalents (GAE)/100 mL, while total flavonoid content (TFC) was determined with sodium nitrite, aluminium chloride and sodium hydroxide and expressed as mg catechin equivalents (CE)/100 mL. AOxA was evaluated by DPPH scavenging activity, Fe(III) reducing power, and Fe(II) chelating capacity. The AMA of BGP polyphenolic extracts was determined using agar disk-diffusion method against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*. The highest TPC and TFC values were found in the ethanolic extract (210.85 mg GAE/100 mL and 119.27 mg CE/100 mL, respectively). Methanolic and ethanolic extracts showed important values for DPPH scavenging activity and Fe(III) reducing power, while ethanolic and acetone extracts presented the highest values regarding Fe(II) chelating activity. Polyphenols extracted in methanol and acetone showed significant AMA, but polyphenols extracted in ethanol showed maximum inhibition zones against all the microorganisms tested; chloroform and aqueous extracts showed less AMAs.

Conclusions

Black grape pomace represents an important source of polyphenols with antioxidant and antimicrobial activity. This work was carried out through Partnerships in priority areas Program-PN II, implemented with the support of MEN-UEFISCDI (Romania), project nr. 149/2014.

FEMS7-1066
Food Microbiology - Part III

CONTRIBUTION OF VEGETABLES AND AGRICULTURAL ENVIRONMENTS TO THE SPREAD OF SHIGATOXIN 2 PHAGES AND THE EMERGENCE OF NEW SHIGATOXIN PRODUCING STRAINS.

P. Quirós[1], *M. Muniesa*[1]
[1]*Universidad de Barcelona, Genetics- microbiology and stadistics, Barcelona, Spain*

Backgrounds

There is a global tendency to eat a healthy diet rich in uncooked fresh vegetables, although consumption of raw products poses a risk of contamination by pathogens. Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens associated with serious human disease. Its principal virulence factor is Shiga toxin (Stx), whose genes are in the genomes of temperate bacteriophages (Stx phages). A relevant number of STEC recent outbreaks are linked to vegetable consumption, as the one in Germany in May 2011. It is unknown the real risk of Stx transduction causing the emergence of new STEC strains in vegetables, particularly when consumed little or no cooked.

Objectives

To evaluate different types of vegetables and agricultural soils for the occurrence, persistence and infectivity of Stx phages and to evaluate *stx* transduction and generation of STEC in these matrices.

Methods

A total of 90 samples (lettuce, cucumbers, spinach, lentil sprouts and soils) were used as matrices. The occurrence of infectious Stx1 and Stx2 phages in these matrices and their persistence at storage temperature (4 and 23ºC) was evaluated. Labelled Stx phages were used to generate *E. coli* lysogens in these matrices.

Conclusions

There is a greater occurrence of Stx2 phages (23.3 % samples) than Stx1 phages (5% samples), particularly in soil. Many Stx phages detected were infectious and persist more than 10 days at storage temperatures. Transduction and generation of STEC lysogens was observed in all matrices. Horizontal transfer of *stx* mediated by Stx phages may give rise to the emergence of new pathogens in vegetable matrices.

FEMS7-0278
Food Microbiology - Part III

EVALUATION OF FRESH SWEETS WITH STAPHYLOCOCCUS AUREUS ENTEROTOXIN GENES A AND B IN KURDISTAN PROVINCE

B. Rahimian Zarif[1], *T. zabihollahi*[1]
[1]*Department of biology - sanandaj branch - Islamic Azad University - sanandaj - Iran, Biology, sanandaj, Iran*

Backgrounds

the main causes of food poisoning in the world are Enterotoxins A and B of *Staphylococcus aureus*. Approximately 20 to 55 percent of healthy adults are infected with this pathogen. Transmission of enterotoxin producer *Staphylococcus aureus* in fresh sweets, through contaminated dairy products or manufacturers, has increased the risk of food poisoning.

Objectives

the aim of the present study was to determine the prevalence of enterotoxin genes (sea) A and (seb) B between *S. aureus* strains isolated from products in several cities of Kurdistan province in 2015**.**

Methods

In cross-sectional study, 43 samples of fresh sweets were collected randomly of 8 cities in Kurdistan province based on the instructions of the Institute of Standard and Industrial Research of Iran and microbiology tests, the presence of *Staphylococcus aureus*, has been evaluated and distribution of sea and seb genes in isolates has been determined by PCR method.

Conclusions

The results have shown that percentage of contaminated fresh sweets with *Staphylococcus* was more than 6.97%. The frequency of enterotoxin sea and seb genes has been evaluated in samples. The contaminated samples with *Staphylococcus aureus* (2.32%) were able to producing enterotoxin A, and non of them were not able to producing enterotoxin B**.** Overall, the results have shown, there is the possibility of contamination fresh sweets with *Staphylococcus* and food poisoning. Pasteurization of dairy products, continuous microbial control fresh sweets and screening of food producer can reduce the risk of staphylococcal food poisoning**.**

FEMS7-2798
Food Microbiology - Part III

CHARACTERISATION OF MULTIDRUG-RESISTANT DIARRHEAGENIC E. COLI FROM BOVINE DAIRY FARMING IN "CASTILLA Y LEON" (SPAIN)

E.A. Rios[1], J. Ramos Pereira[1], J.A. Santos[1], A. Otero[1], J.M. Rodríguez-Calleja[1]
[1]University of León, Department of Food Hygiene and Food Technology, Leon, Spain

Backgrounds

Several studies have showed an increase of multidrug-resistant *E. coli* as a raising concern for public health.

Objectives

This study was undertaken to carry out the characterisation of diarrheagenic *E. coli* obtained from dairy farms placed in the northwest of "Castilla y León" (Spain).

Methods

Fifty-six *E. coli* strains isolated from raw cow's milk (30) and dairy cattle farm environments (26; faeces, water, feed, air and handlers) were tested for biochemical phenotype, genetic profile and susceptibility to 22 antibacterial substances.

Conclusions

Thirty-three (59%) strains were PCR positive for the intimin gene (*eae*), negative for *stx*1 and *stx*2 genes, and lacking pEAF (*bfp*A-), thus considered atypical EPEC (aEPEC). Other virulence factors investigated were *espA*, *espB*, *tir* and *ehxA*. A high frequency (85%) of the tested aEPEC carried all these virulence genes but *ehxA* and only three strains showed these four genes. Nine (28%) aEPEC strains showed antibiotic resistance and six isolates were resistant to three or more antibiotics including ampicillin, cephalothin, cefotaxime, streptomycin, tetracycline, trimethoprim-sulphamethoxazole, sulfonamides, chloramphenicol, and amoxicillin-clavulanic acid.STEC strains (41%), bearing *stx* genes (predominantly *stx*1c), showed also other virulence factors (*hlyA*, *tia* and *subAB*). A high number (52%) of STEC strains exhibited antimicrobial resistance, among which 10 showed multiple resistance with streptomycin/gentamicin/chloramphenicol (4 strains) and ampicillin/cephalothin (4 strains) the most frequent antibiotics included in the resistance profiles. Overall, this study provides further evidence that cow's milk and farm environment are potential sources of both STEC and aEPEC bearing virulence genes and multiple antibiotic resistances which raises concerns for public health through the potential resistance dissemination along food chain.

FEMS7-0648
Food Microbiology - Part III

THE USE OF PEPTIDOGLYCAN HYDROLASES IN FOOD PACKAGING BIOFILMS

T. Robles[1], *M. Quirasco*[1]
[1]*Universidad Nacional Autónoma de México, Faculty of Chemistry, Ciudad de Mexico, Mexico*

Backgrounds

E. faecalis strain F isolated from a Mexican cheese has shown extracellular lytic activity against gram positive and gram negative bacteria such as *S. aureus, Listeria monocytogenes, Micrococcus lysodeikticus, Salmonella enterica* Typhimurium and *Yersinia enterocolitica.* Bacteriolytic activity is associated to different enzymes with N-acetylglucosaminidase activity against bacterial peptidoglycan.

Objectives

The aim of this project was to produce a biofilm with peptidoglycan hydrolases immobilized by entrapment retaining antimicrobial activity against foodborne pathogens.

Methods

To achieve this goal, several mixtures of corn starch with glycerol as plasticizer were tested (thickness, solubility, elongation at break, tensile strength and water vapor permeability). As antimicrobial agents an extract with high concentration of the enzymes produced by *E. faecalis* strain F was immobilized and, lysozyme was tested as well. In the first formulation, the supernatant (previously neutralized) lyophilized was added to the support prior drying; and in the latter, lysozyme was directly added to the cold starch mixture. The catalytic activity of N—acetylglucosaminidase was detected by the hydrolysis of 4-Nitrophenyl N-acetyl-β-D-glucosaminide.

Conclusions

As a result, the film made with 5% (w/v) corn starch and 0.3 (w/w starch) glycerol had the best performance as support for immobilization.
Total activity was determined before and after immobilization and enzyme stability was assessed as well. The free enzyme showed 55% of activity at 4°C, which is important due to the intended use of the film at refrigeration temperature.

FEMS7-0682
Food Microbiology - Part III

DEFINITION OF SAMPLING PROCEDURES FOR COLLECTIVE-EATING ESTABLISHMENTS BASED ON THE DISTRIBUTION OF ENVIRONMENTAL MICROBIOLOGICAL CONTAMINATION ON FOOD HANDLERS, UTENSILS AND SURFACES

D. Rodriguez-Lazaro[1], M. Hernandez[2], J.C. Ortiz[3], G. Fongaro[4], A. Valero[5]
[1]University of Burgos, Biotechnology and Food Science, 9001, Spain
[2]ITACyL, Laboratory of Molecular Biology and Microbiology, Valladolid, Spain
[3]Ayuntamiento de Madrid, Food Hygiene, Madird, Spain
[4]UFSC,
Laboratório de Virologia Aplicada- Departamento de Microbiologia- Imunologia e Parasitologia-, Florianópolis, Brazil
[5]University of Cordoba, Department of Food Science and Technology-, Cordoba, Spain

Backgrounds

Environmental sampling has been identified as an effective procedure to verify correct implementation of food safety control systems in catering establishments. At the same time characterization of microbial distribution of environmental contamination could potentially address effective fit-for-purpose sampling procedures.

Objectives

The study aimed at evaluating the microbiological contamination on food handlers, food-contact utensils and handlers'-contact utensils during food preparation for collective meals in Spain, as well as to determine contamination routes and their relationships between microbial indicators (aerobic mesophilic bacteria, *Enterobacteriaceae*, *Escherichia coli* and *Staphylococcus aureus*). Further, characterization of statistical distributions of microbial contamination and suggestion of sampling procedures were also performed.

Methods

1,202 environmental samples from three types of food catering establishments located in Madrid, Spain were monitored for presence of mesophilic bacteria, *Enterobacteriaceae*, *Staphylococcus aureus* and *Escherichia coli*. Samples corresponded to food-contact utensils, handlers'-contact utensils and food handlers, using 3M™ Petrifilm™ count plates. Contamination routes were identified through the calculation of Spearman correlation coefficients. Further, characterization of statistical distributions of microbial contamination and suggestion of sampling procedures were also performed.

Conclusions

53.0% of the samples were positive for at least one of the bacterial group studied and 328 (27.1%) with 1-15 CFU/plate. *Enterobacteriaceae, E. coli* and *S. aureus* were present in 62.1%, 7.5% and 26.6%, respectively of the food handlers' samples. Contamination routes from food handlers to handlers'-utensils was identified in a bidirectional way, being it subsequently spread to utensils in contact with foods. The microbial distribution affected significantly the number of samples needed to detect positives.

FEMS7-2858
Food Microbiology - Part III

ADAPTATION OF LISTERIA MONOCYTOGENES TO SEVERAL FOOD-GRADE BIOCIDES

C. Rodríguez-Melcón[1], *R. Capita*[1], *C. Alonso-Calleja*[1]
[1]*Universidad de León, Food Hygiene and Technology, León, Spain*

Backgrounds

Biocides are compounds commonly used in the food system to reduce or eliminate both pathogenic and spoilage microorganisms. An increase in biocide tolerance is an important public health issue, as it might be expected to contribute to the increased persistence of pathogens in the food chain.

Objectives

The objective of this study was to determine whether *Listeria monocytogenes* can adapt to growth in increasing concentrations of several biocides commonly used in food processing facilities.

Methods

Four *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 4a and 4b) were tested. The minimum inhibitory concentration (MIC) values of sodium hypochlorite (SHY), peracetic acid (PAA) and benzalkonium chloride (BZK) were established using a microdilution broth method in accordance with the CLSI Standard.

Conclusions

The MIC values (ppm) for *L. monocytogenes* cells ranged from 3,500 to 3,750 (SHY), 1,000 to 1,050 (PAA), and 1.0 to 2.0 (BZK). The cultures exhibited an acquired tolerance to such biocides, especially to SHY and BZK. The maximum concentrations (ppm) of biocides that allowed bacterial growth after several passages through gradually higher concentrations of such compounds ranged from 3,935 to 5,906 (BZK), 750 to 1,125 (PAA), and 1.7 to 8.5 (BZK). The highest adaptive tolerance was showed by *L. monocytogenes* 4a (SHY), 1/2a and 4b (BZK). It is suggested that sub-lethal exposure to food-grade biocides represents a risk for the development of adaptation of *L. monocytogenes* to such compounds. The importance of maintaining higher than MICs of biocides during sanitizing procedures to fight foodborne infections by *L. monocytogenes* is highlighted.

FEMS7-2861
Food Microbiology - Part III

EFFECT OF SEVERAL CONCENTRATIONS OF BIOCIDES ON THE ARCHITECTURE AND VIABILITY OF THE BIOFILMS FORMED BY LISTERIA MONOCYTOGENES

C. Rodríguez-Melcón[1], *C. Alonso-Calleja*[1], *F. Riesco-Peláez*[1], *R. Capita*[1]
[1]*Universidad de León, Food Hygiene and Technology, León, Spain*

Backgrounds

There are a number of circumstances where concentrations of biocides may be at sub-minimum inhibitory concentrations (sub-MICs). A better understanding of the phenomenon of biofilm may assist in the development of control strategies.

Objectives

The effect of sodium hypochlorite (SHY) and benzalkonium chloride (BZK) at different concentrations (0.5MIC, 1MIC, and 1.5MIC) upon the architecture and viability of the biofilms formed by *L. monocytogenes* (strains 2, 6, 7 and 12) was investigated.

Methods

Biofilm images were examined through confocal laser scanning microscopy (CLSM) after staining with SYTO9 and propidium iodide.

Conclusions

Biovolume (mean ± STD in the observation field -14,161 μm^2-) of the biofilms formed in the absence of biocides (control) ranged between 103.928,27 ± 6.730,16 μm^3 (strain 12) and 276.030,94 ± 42.291,89 μm^3 (strain 2). The presence of 0.5MIC of BZK during biofilm formation process increased ($P < 0.05$) the biofilm-forming ability of strains 6, 7 and 12. In contrast, at 1MIC or 1.5MIC, biocides reduced substantially ($P < 0.001$) the ability of *L. monocytogenes* to produce biofilm (biovolume between 28.40 ± 12.40 μm^3 and 266.32 ± 77.24 μm^3). The largest percentages (values higher than 95%) of live (green stained) cells ($P < 0.05$) were observed in biofilms grown in absence of biocides or in presence of 0.5MIC of biocides. In presence of 1MIC or 1.5MIC of biocides, percentages of dead (red) cells ranged between 32.60 ± 21.36% and 87.89 ± 10.29%. The findings of the present study suggest that the use of biocides at sub-inhibitory concentrations could represent a public health risk.

FEMS7-1898
Food Microbiology - Part III

MODELLING AFLATOXIN CONTAMINATION IN DRY-CURED HAM BY APPLYING DATA MINING

B. Peromingo[1], D. Caballero[1], A. Alía[1], V. Bernáldez[1], E. Bermúdez[1], A. Rodríguez[1]
[1]Meat and Meat Products Research Institute, Animal Production and Food science, Cáceres, Spain

Backgrounds

Aspergillus flavus and *Aspergillus parasiticus* are mould species producers of aflatoxins (AFs) and may grow on dry-cured ham during the ripening process. AFs production is mainly influenced by environmental factors, especially water activity (a_w) and temperature (T). Data mining is an emerging technique in modelling which allows predicting data by exploring and analysing large volumes of data.

Objectives

The objective of this study was to predict AFs produced by *A. flavus* and *A. parasiticus* in a dry-cured ham matrix using predictive data mining.

Methods

Lineal model was used to calculate lag phases and growth rates and AFs amounts were quantified by uHPLC–MS/MS. Multiple Linear Regression (MLR) was used for deductive tasks. For predicting AFs, the application of MLR on data mining method is accurate to predict the minimum a_w and T values from which the strains begin to produce AFs (R^2 for AFB_1 and AFG_1 were 0.86 and 0.84, respectively) and the first day the toxin can be synthetised (R^2 for AFB_1 and AFG_1 were 0.88 and 0.87 respectively).

Conclusions

Thus data mining allows determining AFs contamination in dry-cured ham. This predictive model is important in developing prevention strategies to control AFs in dry-cured ham and avoid risks associated with this mycotoxin to consumer´s health.

Acknowledgements: Funded by the Spanish Ministry of Economy and Competitiveness, Government of Extremadura and FEDER (AGL2013-45729-P, GR15108). B. Peromingo is recipient of a pre-doctoral fellowship (BES-2014-069484). Dr. A. Rodríguez is supported by a Juan de la Cierva senior research fellowship (IJCI-2014-20666).

FEMS7-1712
Food Microbiology - Part III

GENOTYPING OF ENTEROTOXIC STAPHYLOCOCCUS AUREUS ISOLATED FROM RAW COW'S MILK AND CHEESES IN POLAND

W. Korpysa-Dzirba[1], J. Rola[1], A. Czubkowska[1]
[1]National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Pulawy, Poland

Backgrounds

In the recent years, the market of raw milk cheeses is growing as such products are considered to be ecological, tasty and they do not have unnecessary additives. Milk without heat treatment used for production of raw milk cheeses may be contaminated by *S. aureus*. Enterotoxic strains of these bacteria can induce the symptoms of staphylococcal food poisoning (SFP).

Objectives

The aim of this study was to detect enterotoxic *S. aureus* recovered from raw cow's milk and estimate their sequence types (ST's).

Methods

Samples of raw cow's milk and cheeses were collected in dairy farms located in the eastern part of Poland. Detection of staphylococcal enterotoxins (SE's) genes and the markers responsible for methicillin resistance were done using multiplex PCRs. The enterotoxic isolates were analysed by MLST technique.

Conclusions

Eleven of 24 (45.8%) and 14 of 29 (48.3%) isolates recovered from raw cow's milk and cheeses, respectively, harboured the SE genes. The combination of *sed*, *ser* and *sej* markers was most often detected (52%), followed by *sep* and a combination of *seg* and *sei* (both 28.0%). Fifteen (60.0%) isolates had more than one SE gene. One (3.4%) *S. aureus* isolated from cheese was methicillin resistant. The sequence types (ST) 97 was often found, however other and some new ST were also identified. These results indicate that the enterotoxic CPS can be often found in raw cow's milk and cheeses and therefore may pose a risk of food intoxication. Estimation of sequence types enables to understand the ways of spreading of the isolates.

FEMS7-1747
Food Microbiology - Part III

ASSESSMENT OF HYGIENIC QUALITY OF RAW GOAT MILK IN POLAND

A. Czubkowska[1], J. Rola[1], W. Korpysa-Dzirba[1]
[1]National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Pulawy, Poland

Backgrounds

The consumption of goat milk and goat milk products has been growing. Goat milk is attractive for consumers due to enhanced nutritional properties and lower allergenic potential in comparison to cow milk. The data on the hygienic quality of goat milk in Poland is limited.

Objectives

The aim of the study was to assess the hygienic quality of raw goat milk.

Methods

A total of 78 samples of raw goat bulk-tank milk from 30 different dairy farms in Poland were collected in 2016. Quality of the samples was determined in relation to the number of *E. coli*, *Enterobacteriaceae*, coagulase-positive staphylococci, total bacteria count and somatic cells count. The analysis were performed according to the standard ISO methods. Furthermore the presence of antimicrobial residues was examined using Delvotest SP NT.

Conclusions

The number of *E. coli* in raw milk samples was determined at an average level of $1.9x10^1$ cfu/ml, *Enterobacteriaceae* - $2,0x10^5$ cfu/ml, coagulase-positive staphylococci - $4.7x10^3$ cfu/ml. The average number of microorganisms in raw milk was $1.0x10^7$ cfu/ml, and the percentage of sample contamination >1500000 cfu/ml accounted for 38.5%. The average of somatic cell count in samples of raw milk was 1 566 000 cells/ml. Antimicrobial residues were detected in 7.8% samples of raw goat's milk. The obtained results indicate that some results of total plate count in raw goat milk exceed the hygiene criterium listed in Commision Regulation 853/2004 and antimicrobial residues are present in milk samples.

FEMS7-1359
Food Microbiology - Part III

IS THE DISTRIBUTION OF LISTERIA SPP. ISOLATED FROM ALL-NATURAL, MIXED SPECIES, PASTURED-RAISED BROILER FARMS RELATED TO DIFFERENTIAL GROWTH?

A. Locatelli[1], M.J. Rothrock - Jr.[1]
[1]USDA-ARS, US National Poultry Research Center, Athens, USA

Backgrounds

Listeria spp. represent an important foodborne pathogen, but relatively little is known about its environmental prevalence on poultry farms. Considering the environmental exposure inherent with pasture-raised production systems, these types of alternative poultry management systems represent an ideal setting to determine environmental *Listeria* spp. diversity and prevalence.

Objectives

Initial surveys of the isolate datasets revealed that across the farms samples *L. innocua* (59%) was found predominantly in feces and soil samples followed by *L. monocytogenes* (33%) and *L. welshimeri* (2%). Based on theses observations, we wanted to evaluate whether the distribution of *Listeria* species evidenced in broiler farms could results from a differential growth in liquid media.

Methods

Four *Listeria* strains isolated from soil were selected including one of each *L. monocytogenes* serogroups and one of *L. innocua*. These strains were inoculated either separately or in mixed culture in Tripticase Soy Broth (TSB) and University of Vermont (UVM) modified *Listeria* enrichment broth at 10^2 and 10^5 cells per ml and incubated for 24-48 hrs at 3 temperatures (20, 30, 42˚C).

Conclusions

Overall, the inoculum concentration and the liquid media have a significant effect on *Listeria* growth at all temperatures. No significant differences were observed between the growths of the three *L. monocytogenes* strains. In UVM media, a significantly shorter lag phase was observed for *L. innocua* compared to *L. monocytogenes* strains for both inoculum concentrations. This difference in the growth dynamic between may help to explain the *Listeria* species recovered from these broiler farms.

FEMS7-2100
Food Microbiology - Part III

FLUORESCENT LABELING OF BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS TO DECIPHER THE FUNCTIONAL ROLE OF ITS EXOPOLYSACCHARIDE COVER

N. Castro-Bravo[1], C. Hidalgo-Cantabrana[1], A. Margolles[1], P. Ruas-Madiedo[1]
[1]IPLA-CSIC, Microbiology and Biochemistry of Dairy Products, Villaviciosa-Asturias, Spain

Backgrounds

The exopolysaccharide (EPS) layer covering the surface of different bacteria, including *Bifidobacterium animalis* subsp. *lactis*, has been associated with the beneficial properties attributable to the producing bacterium. Indeed, EPS are one of the microbial associated molecular patterns that initiate the interaction with the host.

Objectives

Our aim was to obtain different EPS-producing *B. animalis* subsp. *lactis* variants, harboring plasmids with genes coding for fluorescent proteins, to study the role of EPS on the adherence of the producing strain.

Methods

One chromosomally stable EPS-producing variant (S89L strain) was obtained by means of a double crossover marker-less strategy. In the type strain DSM10140 a gene was replaced with another containing a nonsynonymous mutation associated with the ropy phenotype, yielding S89L. Besides, the type DSM10140 strain and the ropy S89L variant were labeled with two plasmids containing genes for mCherry or the green fluorescent proteins, thus yielding four fluorescent strains. These strains were used to check their adhesion to the human intestinal cell line HT29 as well as their capability to form biofilms in different abiotic surfaces (gold, polystyrene and glass) using different methodologies (real time monitoring and end-point crystal-violet staining).

Conclusions

This is the first report on the construction of fluorescent labeled *B. animalis* subsp. *lactis* strains; the qualitative and quantitative fluorescence analyses showed that the ropy S89L strain displayed a reduced capability to adhere to the intestinal epithelial monolayer and to form biofilms under abiotic surfaces. These results underline the usefulness of fluorescent labeling to elucidate the biological properties of bifidobacterial EPS.

FEMS7-2506
Food Microbiology - Part III

MICROBIAL DIVERSITY IN TRADITIONAL RAW EWE'S MILK SERPA CHEESE

G.D.S. Maria Teresa P.[1], M.J. Benito[2], M.D.G. Córdoba[2], C. Egas[3], S. Ruiz-Moyano[2]
[1]Instituto Politécnico de Beja, Escola Superior Agrária de Beja, Beja, Portugal
[2]Instituto Universitario de Investigación en Recursos Agrarios INURA- Universidad de Extremadura, Producción Animal y Ciencia de los Alimentos, Badajoz, Spain
[3]Biocant Park, UCBiotech-CNC, Cantanhede, Portugal

Backgrounds

Serpa is an artisanal ripened Portuguese cheese granted the Protected Designation of Origin (PDO) label. The absence of any standardisation for the thermal process and starter microorganisms means that its quality and characteristics depend mainly on the endogenous flora. Most of the microflora present in raw milk cheese are lactic acid bacteria (LAB), but spoilage and pathogens bacteria may be present too, and this raises the potential of public health risks. Hence, controlling the microbial communities during the production is the main factor to ensure food safety and the sensorial properties of the final product.

Objectives

The aim was to study the fortuitous flora in Serpa cheese to control the microbial communities during its production.

Methods

Microbial diversity of Serpa cheeses from PDO and non-PDO registered industries were evaluated by culture-dependent and independent-methods.

Conclusions

Both approaches demonstrated that microflora mainly corresponded to LAB and to a lesser extent, enterobacteria. The main species identified by conventional 16S rRNA gene sequencing were *Lactobacillus paracasei/casei* in cheese from PDO industries; whereas in non-PDO *Lactobacillus brevis* was highlighted. However, the results obtained by high throughput sequencing analysis showed that although LAB were the main microbial group, *Lactococcus* genus contributing to ~40-60% of the population, followed by *Leuconostoc* and *Lactobacillus.* Among Enterobacteriaceae species identified, *Hafnia alvei* was presented at ~20-30% in all samples, however, *Escherichia coli* was also detected in non-PDO registered industries.

These results suggest the application of an autochthonous starter culture, formed by *Lactococcus* and *Lactobacillus* species, to control the microbial communities during Serpa cheese production.

FEMS7-2737
Food Microbiology - Part III

MICROBIAL DYNAMIC DURING THE SUN-DRYING PROCESS OF FIGS (FICUS CARICA L.) CV. 'CUELLO DAMA BLANCO'

M.D.C. Villalobos[1], M.J. Serradilla[2], S. Ruiz-Moyano[1], A. Martín[1], M.D.G. Córdoba[1]
[1]Instituto Universitario de Investigación en Recursos Agrarios INURA- Universidad de Extremadura, Producción Animal y Ciencia de los Alimentos, Badajoz, Spain
[2]InstitutoTecnológico Agroalimentario de Extremadura INTAEX-CICYTEX, Área de Vegetales, Badajoz, Spain

Backgrounds

The consumption of both fresh and dried figs (*Ficus carica* L.) has recently increased due to their excellent sensorial and nutritional characteristics. Figs are distinguished by their perishability, mainly caused by the microbiological spoilage. The sun-drying process, due to its severe lack of control, can lead to diverse problems such as the fungal growth and subsequent mycotoxin production.

Objectives

The present work studies the microbial population of figs cv.'Cuello Dama Blanco' throughout sun-drying process.

Methods

The population of bacteria, yeasts and moulds were studied at fresh stage as well as in figs undergone a sun-drying process during a period of 15 days. Microorganisms were isolated and identified by sequence of the16S rRNA gene for bacteria and ITS region for fungi.

Conclusions

The results showed that fresh figs revealed a higher microbial population than sun-dried figs. At earlier dried fig developmental stages, microbiological populations were mainly dominated by the bacteria species *Pseudomonas gessardii* followed by *Pantoea agglomerans*, while among fungal population highlighted the yeasts species *Aureobasidium pullulans* followed by *Cryptococcus adeliensis, Hanseniaspora uvarum* and *Torulaspora delbrueckii* and a wide diversity of moulds such as *Cladosporium cladosporioides, Alternaria alternata, Cladosporium macrocarpum, Penicillium corylophilum* and *Penicillium brevicompactum*. Notwithstanding, with the sun-drying treatment, bacteria and yeasts disappeared and moulds were clearly the main microbial group with variation in the species identified. In addition to the species previously mentioned on fresh figs, other species such as *Aspergillus fumigates, Penicillium citrinum* and *Penicillum purpurogenum* were also found on sun-dried figs, while *Alternaria alternata* and *Cladosporium macrocarpum* were not detected.

FEMS7-0957
Food Microbiology - Part III

COMPARATIVE GENOMICS AND TRANSCRIPTOMICS OF THIOL-RELEASE PATHWAY GENES IN WINE YEASTS AT FERMENTATION CONDITIONS

J. Ruiz[1], I. Belda[1], C. Barbosa[2], E. Navascués[3], D. Marquina[1], A. Mendes-Ferreira[2], A. Santos[1]
[1]Faculty of Biology- UCM, Microbiololgy, Madrid, Spain
[2]BioISI - Biosystems & Integrative Sciences Institute Universidade de Trás-os-Montes e Alto Douro, Escola de Ciências da Vida e Ambiente, Vila Real, Portugal
[3]Agrovin- S.A., Research & Development, Ciudad Real, Spain

Backgrounds

Varietal thiols (4-mercapto-4-methylpentane-2-one (4-MMP) and 3-mercapto-hexanol (3-MH)) play a key role in the characteristic passion-fruit aroma of some white grape varieties, however they are often present as non-volatile, odourless, conjugated forms in grape juice. Yeasts uptake the cysteinylated precursors (cys-4MSP and cys-3SH) through the general oligopeptide transporter (*GAP1*) and latter cleavage them by the action of β-lyase enzymes (*IRC7)*, releasing the correspondent volatile thiols. Nitrogen Catabolite Repression (NCR) regulates both genes, resulting in a low thiol release in most *Saccharomyces cerevisiae* strains.

Objectives

This work aims to evaluate the influence of nitrogen availability on the expression of thiols release-related genes (mainly *GAP1* as transporter and *IRC7* as β-lyase) in *S. cerevisiae* strains and of the correspondent orthologous genes in *Torulaspora delbrueckii* strains (non-conventional yeast of enological interest).

Methods

Fermentation assays were performed in synthetic grape juice under two nitrogen condition, low (LN) and high concentration (HN). Yeast growth and nutrient consumption were monitored throughout fermentations. RNA samples were obtained at 24h (nitrogen depletion in LN) and 96h (nitrogen starvation in LN) for *IRC7* and *GAP1* expression analysis by qRT-PCR.

Conclusions

Intraspecific differences were found within the *S. cerevisiae* strains regarding *IRC7* and *GAP1*expression levels irrespective of the nitrogen condition. Also, *T. delbrueckii* showed notable physiological differences from *S. cerevisiae* strains in both nitrogen assays. Our results, suggest that in *T. delbrueckii* both genes are differentially regulated by nitrogen, which is line with the increased volatile thiol release previously observed in natural must fermentations under non-limiting nitrogen conditions.

FEMS7-0214
Food Microbiology - Part III

EIIANTR PLAYS A KEY ROLE IN SALMONELLA FITNESS AND VIRULENCE VIA 1,2-PROPANEDIOL METABOLISM

S. Ryu[1], *W. Yoo*[1], *H. Yoon*[2]
[1]*Seoul National University, Department of Agricultural Biotechnology, Seoul, Republic of Korea*
[2]*Ajou University, Department of Molecular Science and Technology, Suwon, Republic of Korea*

Backgrounds

The nitrogen-metabolic phosphotransferase system (PTS^{Ntr}) consists of EI^{Ntr}, NPr, and $EIIA^{Ntr}$ (encoded by *ptsP*, *ptsO*, and *ptsN* respectively). Due to the location of *ptsO* and *ptsN* downstream of *rpoN* in the same operon, this system has been postulated to be involved in nitrogen metabolism. However, a specific substrate transferred by PTS^{Ntr} is yet to be determined. Instead, a number of studies demonstrate that PTS^{Ntr} exerts multifaceted roles as a regulatory system in diverse bacteria.

Objectives

In order to understand roles of PTS^{Ntr} in *Salmonella enterica* serovar Typhimurium, bacterial transcriptome of a Δ*ptsN* strain was analyzed.

Methods

S. Typhimurium SL1344 and its isogenic mutant strain, Δ*ptsN*, were subjected to RNA sequencing. The results of transcriptome analysis were evaluated using qRT-PCR, Western-blot analysis, and Invasion assay. Total RNAs were isolated from *Salmonella* strains at mid-log phase in LB medium. RNA sequencing was performed using the Illumina GAII (Illumina).

Conclusions

$EIIA^{Ntr}$ positively regulated the expression of genes involved in Vitamin B_{12} synthesis and 1,2-propanediol metabolism, whereas negatively controlled the expression of *Salmonella* pathogenicity island 1 (SPI-1), which is required for bacterial invasion into host cells. In accordance with this observation, *Salmonella* lacking $EIIA^{Ntr}$ was more competent to invade host cells than wild-type *Salmonella* when supplemented with 1,2-propanediol as carbon and energy sources. These results suggest that *Salmonella* exploits $EIIA^{Ntr}$ as a key player to modulate proliferation and invasion in the host intestine.

FEMS7-0448
Food Microbiology - Part III

EFFECT OF THE PH ON THE ANTIVIRAL ACTIVITY OF THE (-)-EPIGALLOCATECHIN GALLATE

G. Sánchez[1], . Walter Randazzo[2], I. Falcó[2], L. Gómez-Mascaraque[1], A. López-Rubio[1], R. Aznar[2]
[1]Instituto de Agroquímica y Tecnología de los Alimentos,
Tecnologías de Conservación y Seguridad Alimentaria, Paterna, Spain
[2]University of Valencia, MIcrobiology and Ecology, Paterna, Spain

Backgrounds

Epigallocatechin gallate (EGCG), a flavonoid from green tea, is said to have extensive antimicrobial activity in a wide range of food spoilage or pathogenic fungi, yeast and bacteria.

Objectives

In this work, the antiviral activity of EGCG was assessed against hepatitis A virus (HAV) and murine norovirus (MNV), a human norovirus surrogate, at different temperatures, contact times and pH conditions.

Methods

Antiviral activity of EGCG was evaluated by cell-culture methods, viability RT-qPCR and RT-qPCR at different exposure times at 4, 25 and 37 °C. In order to elucidate the antiviral effect of green tea catechins and their derivatives, EGCG solutions (2.5 mg/mL) were prepared in PBS at the different pHs and analysed through HPLC-MS.

Conclusions

EGCG was effective in reducing the titers of HAV and MNV in a dose-dependent manner at neutral pH and 25 and 37 ºC, while no effect was reported at 4 ºC. Furthermore, results also revealed that EGCG was effective inactivating MNV and HAV at neutral and alkaline pH but was ineffective at pH 5.5. Results from cell-culture and viability RT-qPCR assays indicated no differences and therefore it suggests that EGCG did not dramatically affect the viral capsid, which instead might suffer subtle alterations of proteins. Moreover, HPLC/MS analysis of catechin solutions at different pHs indicated that antiviral activity was most likely due to catechin derivatives rather than EGCG itself, given the evolution of these compounds at the various pH conditions tested. These findings suggest that green tea catechins have the potential to be used as natural antimicrobials to control enteric virus contamination.

FEMS7-1632
Food Microbiology - Part III

VIABILITY RT-QPCR FOR ASSESSING ENTERIC VIRUS INFECTIVITY IN SHELLFISH

G. Sánchez[1], W. Randazzo[2], F. Le Guyader[3], R. Aznar[2]
[1]Instituto de Agroquímica y Tecnología de los Alimentos, Tecnologías de Conservación y Seguridad Alimentaria, Paterna, Spain
[2]University of Valencia, Microbiology and Ecology, Paterna, Spain
[3]IFREMER, Laboratoire de Microbiologie, Nantes, France

Backgrounds

The incidence of human noroviruses (NoVs) and hepatitis A virus (HAV) outbreaks due to contaminated food or water shows a rising trend in the last years. Since enteric virus detection is based on RT-qPCR, differentiation between infectious and inactivated enteric viruses is a major challenge to stablish the real health risk associated to contaminated food.

Objectives

To explore the potential of new intercalating dyes to differentiate between infectious and thermally inactivated NoVs and HAV suspensions using the RT-qPCR assay proposed in the ISO 15216 and to assess its applicability in shellfish samples.

Methods

Initially, PMA and PEMAX (GenIUL), and PMAxx (Biotium) at a final concentration of 50 µM were compared by treating infectious and thermally-treated (99 ºC for 5 min) NoVs (GI and GII) and HAV suspensions in PBS. Then, mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*) concentrates were prepared according to ISO 15216. After the PMAxx pretreatment, the RNA was extracted and quantified by RT-qPCR. Additionally, bio-accumulated and naturally contaminated oysters were analysed to verify the pretreatment performance.

Conclusions

PMAxx performed better than PMA and PEMAX since it removed the RT-qPCR signal from thermally inactivated NoVs and HAV. In shellfish, pretreatment with 50 µM PMAxx and 0.5% Triton X-100 completely removed the RT-qPCR signal from thermally inactivated HAV and NoV GII, but not from NoV GI. On bio-accumulated oysters, this pretreatment totally removed the signal of both thermally inactivated NoVs.

According to the results, the PMAxx-Triton pretreatment successfully discriminates between infectious and thermally inactivated enteric viruses, in shellfish, and it can be easily incorporated to the ISO procedure for virus detection (ISO 15216).

FEMS7-0894
Food Microbiology - Part III

LACTOBACILLI ENUMERATION AND BIOCHEMICAL IDENTIFICATION OF ISOLATES OF TOP YOGURT BRANDS

G. Satchanska[1], N. Dimov[1], V. Ahad[1], A. Doycheva[1], D. Ilin[1]
[1]New Bulgarian University, Natural Sciences, Sofia, Bulgaria

Backgrounds

Lactic acid bacteria are pronounced probiotics. They strongly improve the human immune system and produce beneficial for the human health vitamins. Lactobacilli shows strong bactericidal and antifungal activity. Due to synthesis of many enzymes involved in degradation of toxic substances yogurt is known as a food favourable to higher longevity.

Objectives

Six top brands of 3.6 % cow yogurt available at the bulgarian market were examined.

Methods

Lactobacilli count was enumerated in 1 g yogurt *via* the Koch's method. Incubation was performed on MRS media at 45^{o} C for 24 h. Lactobacilli were isolated on enriched with skim milk MRS agar. API 50 CH kit with 50 different metabolites: glycerol, erythrol, D-and L-arabinose, D-ribose, D- and L-xylose, D-adonitol, methyl-beta-D-xylopiranoside, D-galactose, D-glucose, D-fructose, D-mannose, D-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-alpha D-mannopyranoside, methyl-alpha D-glucopyranoside, N-acetylglucosamin, amygdalin, arbutin, esculin/ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melizitose, D-rafinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium guconate, potassium 2-ketoguconate, potassium 5-ketoguconate ribose was used for examining the isolates' biochemical activity.

Conclusions

Our results revealed the following number of lactobacilli per gram yogurt: Elena yogurt - 0,7x10^{6}, Vereia yogurt – 2.6x10^{6}, Na baba yogurt - 3.4x10^{6}, Rodopeia yogurt - 4x10^{6}, Parshevitza yogurt - 2.8x10^{7}, LB yogurt - 7.1x10. All examined samples covered the EU standard except one sample, which was close to standard. Isolated lactobacilli were identified as *Lactobacillus bulgaricus* ssp. *delbrueckii* being positive in degradation of only 4 out of 50 metabolites: D-glucose, D-fructose, D-mannose and D-lactose.

FEMS7-1581
Food Microbiology - Part III

QUALITATIVE AND QUANTITATIVE CHARACTERISTIC OF YEASTS DURING SAUERKRAUT FERMENTATION OF DIFFERENT VARIETIES

P. Satora[1], M. Skotniczny[1], S. Strnad[1], M. Cioch[1]
[1]University of Agriculture in Krakow,
Department of Fermentation Technology and Technical Microbiology, Kraków, Poland

Backgrounds

Sauerkraut is the most important vegetable silage produced in Europe. It is obtained traditionally by spontaneous fermentation of cabbage with indigenous microbiota mainly lactic acid bacteria but also yeasts and fungi. The quality of sauerkraut depends mainly on microbiota present on the leaves surface of raw cabbage. The correct sequence of microorganisms is essential in achieving a stable product with flavor and aroma typical of sauerkraut.

Objectives

The aim of this study was to characterize biodiversity of yeasts present during fermentation of 8 varieties of cabbages.

Methods

Yeast were enumerated using WL Nutrient Agar with Chloramphenicol. Isolates were identified and classified by PCR-RAPD and –RFLP methods. PCR products of the 5.8S ITS domain of selected isolates per distinct RFLP pattern were gel purified (QIAquick PCR purification kit; QIAGEN), and both DNA strands were directly sequenced (Macrogen; http://www.macrogen.com). BLAST searches were performed with the NCBI/GenBank database, and the ClustalX software (http://www-igbmc.u-strasbg.fr/BioInfo) was used to construct multiple-sequence alignments.

Conclusions

The yeasts were present only during first three days of fermentation. Kamienna Glowa cabbage were characterized by the highest level of yeasts ($2{,}98 \cdot 10^3$ CFU/g), the lowest – Cabton (4 CFU/g). From the beginning of fermentation the amount of yeasts gradually decreased. 73 isolates were used for PCR-RAPD analysis. 25 different patterns were found. *Pichia* genus representatives, mainly *Pichia membranifaciens* predominated among isolates, with lower amounts of *Candida*, *Geotrichum* and *Rhodotorula* strains.

This research was funded by Polish National Science Center as a part of the project no. DEC-2014/15/B/NZ9/04527.

FEMS7-2108
Food Microbiology - Part III

ELECTRONIC MICROSCOPY BRINGS NEW KNOWLEDGE ON THERMORESISTANCE OF CLOSTRIDIAL ENDOSPORES WHEN HURDLE TECHNOLOGY IS COMBINED TO THERMAL TREATMENTS

T. Savard[1], J. Barrette[1], M.R. Zareifard[1], S. Grabowski[1], D. Chabot[2]
[1]Food Research and Development Centre, Agriculture and Agri-Food Canada, St-Hyacinthe, Canada
[2]Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, Canada

Backgrounds

Hurdle technologies which mean a combination of treatments to enhance food safety are nowadays largely used by food processors. Scientists have validated multiple mild combinations to control the most common food-borne pathogens, *Listeria, Salmonella* and *Escherichia* while *Clostridium botulinum* has been almost forgotten since it requests drastic thermal treatments.

Objectives

However, harsh treatments affect quality and nutritional value of canned foods. The goal of this study was to decrease the intensity of thermal treatments by combining other hurdles like organic acids.

Methods

An experimental design has been developed to verify the effects of selected processing parameters (pH, acidulants, vegetables, and temperature) on the thermal resistance (D and z-values) of *Clostridium sporogenes* PA3679, *Clostridium botulinum* 62-a, PC0101AJ0 and 13983B and scanning/transmission electronic microscopy has been used to measure changes on spore's components.

Conclusions

The results indicate that a very mild acidification from natural pH (5.8) to pH of 4.8 with organic acids ensure a significant reduction of D-value for all tested species. However, those results also show a significant discrepancy between the measured thermal resistance of the strains and the expected ones as 6 log of *C. botulinum* were totally destroyed at 108°C in 30 sec. in acidified beans. With the help of electronic microscopy, it appears that the core, the protein coat, the cortex and other components are affected differently by thermal treatments combined with organic acids. This study brings therefore, a significant new knowledge to control the safety of food and may help to reduce harsh treatments in the canning industry.

FEMS7-2016
Food Microbiology - Part III

MICROBIOLOGICAL EVALUATION OF GROUND BEEF MARKETED IN BUTCHER SHOPS SUPERMARKETS IN THE SOUTHERN REGION OF CUIABA-MT

M.H. Scabora[1], C.C.P. Barros[2], A.T.D.S. Faustino[1], E.D.A. Silva[1], K.R. Rosa[3], P.A. Testa[1]
[1]Faculdade de Tecnologia SENAI Mato Grosso, Educação, Cuiabá - Mato Grosso, Brazil
[2]FATEC SENAI MT, Educcation, Cuiabá, Brazil
[3]Instituto Federal de Educação- Ciência e Tecnologia de Mato Grosso, Tecnologia de Alimentos, Instituto Federal de Educação- Ciência e Tecnologia de Mato Grosso-, Brazil

Backgrounds

Fresh ground meat usually has a higher microbial count than whole pieces, because there is greater contact due to its processing, contributing to the development of microorganisms.

Objectives

The objective of this study was to evaluate the microbiological and physico-chemical quality of the ground beef sold in supermarket butchers in the southern region of Cuiabá-MT.

Methods

Six supermarkets (A, B, C, D, E, and F) were selected in the southern region of the city of Cuiabá - MT, where a sample was collected from each point, and the analyzes were carried out in triplicate. The samples had the average weight of 250 g, and these were already milled on the tray of the butcher's shop, as soon as the supermarkets opened. They were packed in a thermal box to maintain the temperature and characteristics of the product. The temperature of the samples was measured using a digital spit thermometer (-50 ºC to +300 ºC), shortly after purchase. Microbiological and physico-chemical analyzes were carried out as soon as the samples arrived at the Laboratory of Food Microbiology and Physical Chemistry of the SENAI Mato Grosso Technology College.

Conclusions

Taking into account the results obtained, it is possible to conclude that, in relation to the microbiological quality, all the analyzed samples do not comply with the current legislation RDC 12 of 2001. There is a need to intensify the inspection with the objective of improving sanitary education and the awareness of the Manipulators at the butcher and supermarket managers.

FEMS7-1642
Food Microbiology - Part III

THE HONEY FROM APIS MELLIFERA SSP. SICILIANA TO TACKLE LISTERIA MONOCYTOGENES ISOLATED FROM FOOD

D. Schillaci[1], M.G. Cusimano[1], S. Migliore[2], S. Sapienza[1], V. Arizza[1], M. Vitale[2]
[1]University of Palermo, STEBICEF, Palermo, Italy
[2]Istituto Zooprofilattico Sperimentale Sicilia, A. Mirri, Palermo, Italy

Backgrounds

Listeria monocytogenes is an important foodborne pathogen and its growth as biofilm, intrinsically resistant to several antimicrobial treatments and sanitization procedures, is cause of contamination and environmental persistence in the food industry.

Objectives

To find alternative treatments to the use of antibiotics and disinfectants on farms and industrial setting, we evaluated the efficacy of honey (thistle flowers) from the *Apis mellifera* ssp. *siciliana* on the growth and persistence as a sessile community (biofilm) of some isolates of *L.monocytogenes*.

Methods

The ability to grow as biofilms of twenty-five *L. monocytogenes* isolated, supplied by the Italian Reference Laboratory (IRL), was assessed by using flat-bottom 96-wells plates at 37°C in TSB medium added with 2% glucose and staining the biomass with crystal-violet 0.1%. The ability to produce biofilms ranged from a higher value of optical density (OD) at 600 nm of 0.888 to a lower of 0.148. Antimicrobial properties of *siciliana* bee honey were assessed on isolates and reference strains of *L. monocytogenes*. All tested isolates were susceptible to honey at least to MIC values at the maximum screening concentration of 50% w/v. However MIC values of 30 (% w/v) of honey were obtained for some strains. The anti-biofilm properties of honey were assessed at a concentration of 50% w/v against the best biofilm producers strains (OD ranging from 0.888 to 0.593) and we found inhibition percentages ranging from 73 to 55.5.

Conclusions

The obtained data are promising because indicate a way to replace or reduce conventional disinfectants or antibiotics from food processing facilities.

FEMS7-0058
Food Microbiology - Part III

EFFECTS OF GAMMA IRRADIATION ON COLOR AND MICROBIAL LOAD OF SAFFRON FROM DIFFERENT AREAS OF IRAN

M. Seyhoon[1], M. Barzegar[2], M.A. Sahari[3], G. Khoshpouri[1]
[1]NSTRI, Research School of Radiation Applications, Tehran, Iran
[2]Tarbiat Modares University,
Department of Food Science and TechnologyResearch School of Radiation Applications, Tehran, Iran
[3]Tarbiat Modares University, Department of Food Science and Technology, Tehran, Iran

Backgrounds

Saffron (Crocus sativus L.), from the Iridaceae family, is the world·s most expensive spice. Gamma irradiation is a technique which uses are gradually increasing worldwide, with positive effects on preventing decay by sterilizing the microorganisms and by improving the safety without compromising the nutritional properties and sensory quality of the foods.

Objectives

In this research the effects of gamma irradiation on microbial load, shelf life and some other characteristics of Iranian native saffron from defferent regions of Khorasan province were studied.

Methods

Saffron samples were collected from 3 regions of Khorasan - Iran including Ghaenat, kalat and Torbate heydariyeh and packaged in poly-ethylene bags. The samples were irradiated at doses of 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 kGy then stored at room temperature for 60 days. Chemical characteristics such as colour indices a *, b *, L by Hunter lab and the microbial load were investigated.

Conclusions

Changes in colour indices showed direct relation with the intensity of irradiation dose. The color intensity was increased by increasing doses and it was maintained after 2 months storage in Ghaenat samples. In addition with increasing irradiation dose the amounts of ΔEh were little in Ghaenat samples

The un-irradiated saffron samples showed vast amounts of microbial contamination. After 4 kGy radiation spore forming bacteria was removed completely and aerobic mesophyll, *Enterobacteriaceae*, *coliforms* and fungi counts were decreased, significantly ($p<0.05$). The lowest microbial load was belonged to Ghaenat, kalat and Torbate Heydariyeh regions, respectively. *E.coli* was observed in no samples.

Generally, in all samples from 3 regions, by considering the changes in color parameters and decreasing microbial load the dose of irradiation 4 kGy was the optimum and suggested dose.

FEMS7-1734
Food Microbiology - Part III

UN-SHIELDING EXTRACELLULAR MATRIX OF BIOFILM FORMING BACTERIA PROVIDES NOVEL MEAN TO IMPROVE DAIRY PRODUCTS MICROBIAL QUALITY

M. Shemesh[1], N. Ben-Ishay[1,2], D. Inbar[1,3], R. Reifen[2], D. Steinberg[3]
[1]Agricultural Research Organization ARO The Volcani Center, Food Quality and Safety, Rishon LeZion, Israel
[2]The Hebrew University- The Robert H. Smith Faculty of Agriculture- Food and Environment, Biochemistry- Food Science and Nutrition, Rehovot, Israel
[3]The Hebrew University-Hadassah, Institute of Dental Sciences, Jerusalem, Israel

Backgrounds

Microbial damages caused by biofilm-forming bacteria in the dairy industry are a fundamental threat to safety and quality of milk products. *Bacillus* species, which are major contaminants of dairy products, can form structured multicellular communities known as biofilms on contact surfaces as well as within the milk products themselves.

Objectives

We investigate the role of the extracellular matrix of *Bacillus subtilis* produced through biofilm formation in bacterial survival during milk processing. Furthermore, we develop novel approach of mitigating biofilm formation within milk towards improving dairy products quality and safety.

Methods

Using molecular genetics and food microbiology methodology, we found that the extracellular matrix produced by *B. subtilis* during biofilm formation within milk have a major role in bacterial survival during the milk processing. Noticeably, the mutant strains unable of matrix production were found to be hypersensitive to milk processing procedures such as heat pasteurization. This finding indicates that extracellular polymeric substances serve as a protecting material for biofilm forming bacteria and by un-shielding this protective structure would lead to increasing sensitivity of bacterial cells to stressful environments encounter during milk processing. Therefore, we further aimed to mitigate biofilm formation using the ability of divalent ions such as Mg^{2+} and Ca^{2+} of blocking matrix production by *Bacillus* species. Thus, our further observations indicate about hypersensitivity of bacterial cells, in the presence of these divalent ions, to heat pasteurization undertaken during milk processing.

Conclusions

We suggest that un-shielding biofilm forming bacteria from the extracellular matrix may provide novel mean to improve dairy products microbial quality.

FEMS7-2623
Food Microbiology - Part III

ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS FAMILY OF LAMIACEAE LINDL

O. Shemshura[1], *A. Sadanov*[1], *N. Bekmakhanova*[1], *S. Aitkeldieva*[1], *K. Iskandarova*[1]
[1]*Institute of Microbiology and Virology, Department of Education and Science, Almaty, Kazakhstan*

Backgrounds

At present intensive development of research of green technologies to produce antifungal agents on the basis of green plants, or aqueous (organic) extracts. Plant species of the family *Lamiaceae Lindl*. have a wide range of biologically active substances and have great potential as a basis for the creation of biological products for plant protection.

Objectives

Investigation of antifungal activity of the extracts of plants of the family *Lamiaceae Lindl*. against pathogens of fungal diseases of soybeans and tomatoes. Identify of biologically active components.

Methods

The objects of study were phytopathogens *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus niger*, isolated from infected soybean plants and tomatoes and ethanol extracts of the plant species: *Monarda citriodora*; *Hyssopus officinalis*; *Satureja hortensis*; *Ocimum basilicum* of the family *Lamiaceae Lindl*. Antifungal activity of the ethanol extracts was determined by agar diffusion method. Biologically active components in the extracts were detected by biochemical methods.

Conclusions

All studied extracts inhibited the growth of the pathogenic fungus *Alternaria alternata*, zones of inhibition of growth was between 18 to 24 mm, depending the species of plant. The growth of *Fusarium oxysporum* was inhibited by *Monarda citriodora* and *Ocimum basilicum* (the inhibition halo of the pathogen were 14 mm). In the *Hyssopus officinalis* extract were found components - analogs of ciklopenine and verrukozine. In the extracts of the *Monarda citriodora* and *Ocimum basilicum* detected benzoic acid. In the extract of *Satureja hortensis* is found protocatechuic acid. The results can be used in the development and application of green technologies to protect plants from fungal infections.

FEMS7-2634
Food Microbiology - Part III

EFFECT OF ARACHIDONIC ACID OF THE FUNGUS MORTIERELLA SP. ON THE SYNTHESIS OF MYCOTOXINS BY FUNGI OF THE GENUS FUSARIUM, ALTERNARIA AND BOTRYTIS

O. Shemshura[1], A. Sadanov[1], N. Bekmakhanova[1], G. Mombekova[1]
[1]Institute of Microbiology and Virology, Department of Education and Science, Almaty, Kazakhstan

Backgrounds

In recent years, promising is the use of arachidonic acid from fungi of the genus Mortierella as an inducer of plant resistance to phytopathogenic fungi. Preparations of this type are of low toxicity to a useful fauna on the effectiveness of action on the phytopathogens are not inferior to the nature of the chemical fungicides and lower cost and low consumption rates make them the use of ecologically beneficial. Investigation of the mechanism of action of arachidonic acid on the phytopathogenic fungi is of great interest.

Objectives

to investigate the influence of arachidonic acid on the synthesis of mycotoxins by phytopathogenic fungi

Methods

Effect of arachidonic acid of the fungus Mortierella sp. the change in the qualitative composition of mycotoxins extracts phytopathogenic fungi of the genus Fusarium, Alternaria, Botrytis was investigated by the biochemical method

Conclusions

It has been established that arachidonic acid, isolated from of the fungus Mortierella sp. affects synthesis of toxic metabolites by pathogenic fungi genera Alternaria, Fusarium, Botrytis. Thus, the phytopathogenic fungi unable to synthesize some mycotoxins if the arachidonic acid included into the culture medium. On the medium without arachidonic acid (control), the synthesis of these mycotoxins is detected. For example, the fungus Fusarium oxysporum has lost the ability to synthesize verrukofortin and rukofortin; fungus Alternaria alternata is not able to synthesize fellutanin, rukofortin and regulozuvin; Botrytis cinerea has lost the ability to synthesize regulozuvin B verrukofortin and verrukozin.

FEMS7-1798
Food Microbiology - Part III

HELICOBACTER PYLORI INFECTION IN MOTHERS MODIFIES FECAL MICROBIOTA IN THEIR NEWBORNS ACCORDING TO THE MODE OF DELIVERY

H. Shin[1], C. Hernandez[2], P. Troncoso[2], M. Ortiz[2], M. Vera[2], G.M. Dominguez-Bello[3], P.R. Harris[2]
[1]Sejong University, Food Science and Biotechnology, Seoul, Republic of Korea
[2]Pontificia Universidad Católica de Chile School of Medicine,
Pediatric Gastroenterology and Nutrition, Santiago, Chile
[3]New York University School of Medicine, Medicine, New York, USA

Backgrounds

Interactions of resident intestinal microbes with the mucosal surface play important roles in normal intestinal development. Long-standing infections such as gastric *H. pylori* modify the gastric microbiota and might modify fecal microbiota composition.

Objectives

To evaluate the fecal microbiota of mother-child pairs and its relation to *H. pylori* status.

Methods

A total of 22 mother-child pairs were recruited and we took one stool sample of the mothers before hospital discharge and one stool sample of the newborns at home (15 days old). Maternal *H. pylori* status was evaluated by antigen detection. The V4 region of the 16S rRNA gene was sequenced using Illumina MiSeq platform. Sequences were analyzed using the QIIME pipeline.

Conclusions

Fifty percentage of mothers (11/22) have positive *H. pylori* status. Thirteen babies were vaginally delivered and 9 were born by C-section. All babies were fully breastfed. *H. pylori* was not detected in the feces of newborns. The analyses showed that there were differences in the structure of the microbiota by maternal *H. pylori* status only in infant feces born vaginally (PERMANOVA, p=0.01). Although with similar bacterial alpha diversity level, infants born vaginally to *H. pylori*-infected mothers had higher abundance of *Enterobacteriaceae* and *Veilonella* (LEfSe analysis, LDA>3.0-fold). Maternal *H. pylori* status affects the fecal microbiota composition in babies born by vaginal delivery, but not in babies born by C-section. The results suggest that the effect of the maternal *H. pylori* on the infant fecal microbiota is mediated by the acquired vaginal microbiota at birth.

FEMS7-0331
Food Microbiology - Part III

CASE STUDY OF SHELF LIFE OF SWEET-POTATO KING'S CAKE

L. Silva[1], M. Sale Gomes[1], F. Martins[2], A.M. Cavaco[3], M.D. Antunes[4], M.L. Faleiro[5]
[1]Duas Siglas, Research, Faro, Portugal
[2]FILIPE MARTINS-GESTÃO DE NEGÓCIOS- UNIPESSOAL LDA, Bakery, Olhão, Portugal
[3]Universidade do Algarve, Center for Electronics- Optoelectronics and Telecommunications, Faro, Portugal
[4]Universidade do Algarve, MeditBio- Centro para os Recursos Biológicos e Alimentos Mediterrânicos, Faro, Portugal
[5]Universidade do Algarve, Center for Biomedical Research, Faro, Portugal

Backgrounds

The shelf life of a food product is dictated by the time interval during which maintains its organoleptic properties. These properties can be lost due to several factors including the action of spoilage microorganisms and alteration of physico-chemical characteristics. Moreover the growth of foodborne pathogens must be limited assuring food safety to consumers.

Objectives

In this study, the quality of sweet-potato king's cake over storage at two different temperatures (room temperature ~24 ºC and 4 ºC) during 43 days was determined.

Methods

The pH value, texture, water activity and sugar concentration (glucose, fructose and sucrose) were evaluated. The microbial counts included aerobic mesophilic microorganisms, molds and yeasts, and *Enterobacteriaceae*. For the first 14 days, in a weekly basis, a sensory analysis was also conducted with a taste panel representative of the common consumer.

Conclusions

Results showed that there were no significant differences between the two storage temperatures after 14 days for all tested parameters. Through the remaining days it was observed a tendency to dryness by increasing texture and decreasing of water activity values. In terms of microbiological quality the mesophilic microorganisms, *Enterobacteriaceae* and molds and yeasts after 14 days of storage were within acceptable levels. However, at 28 days for cakes stored at room temperature microbial growth was detected. In conclusion, the limit interval ("best before") for consuming this type of cake should be considered the 14 days after baking.

FEMS7-2198
Food Microbiology - Part III

THE IMPACT OF POTASSIUM METABISULFITE AND ELECTROMAGNETIC IRRADIATION ON LACTOBACILLUS PARACASEI SPP. PARACASEI AT DIFFERENT PHS OF GROWTH MEDIA

D. Soghomonyan[1], A. Trchounian[2]
[1]Yerevan State University- Research Institute of Biology, Biochemistry- Microbiology and Biotechnology, Yerevan, Armenia
[2]Yerevan State University, Biochemistry- Microbiology and Biotechnology, Yerevan, Armenia

Backgrounds

Certain strains of lactic acid bacteria (LAB) could contaminate wines and are known as wine spoilage bacteria. Optimal pH for growth of LAB is 6.5, but the most wines having a pH between 2.9 and 3.9. Probably the combined effects of wine preservative potassium metabisulfite ($K_2S_2O_5$) and electromagnetic irradiation (EMI) could suppress growth of LAB.

Objectives

The aim of this study was to investigate the combined effects of low intensity high frequency EMI (after 1 h irradiation) at 51.8 and 53 GHz frequencies (the flux capacity of 0.06 mW/cm^2), and different concentrations (30 and 300 mg/l) of $K_2S_2O_5$ on growth and survival of *Lactobacillus paracasei spp. paracasei* at pH 6.5 and 3.7.

Methods

For revealing these effects,the growth specific rate (μ) and colony forming units (CFU) number were determined for irradiated and non irradiated bacteria.

Conclusions

Revealed, that at pH 6.5, 300 mg/ l $K_2S_2O_5$ decreased μ ~1.5 and CFU in ~1.25 fold, and combination with EMI at both frequencies suppressed μ ~2 and 2.4 and CFU ~1.5 and 1.7 fold, respectively, while 30 mg/l had no suppressive effect on the growth of bacteria. The effects were stronger at pH 3.7: 300 mg/ l $K_2S_2O_5$ totally suppressed μ and CFU of irradiated and non-irradiated cells. The 30 mg/l $K_2S_2O_5$ decreased μ ~2.2 and CFU 10^2 fold and the combination with EMI at both frequencies decreased μ ~3.4 and CFU 10^3 times compared with non-irradiated samples.

It is assumed that low pH conditions and EMI enhanced the antibacterial effects of $K_2S_2O_5$ on LAB.

FEMS7-0779
Food Microbiology - Part III

A REDUCTION IN LACTOSE IN PROBIOTIC YOGURT USING LACTOBACILLUS PLANTARUM LN4 WITH HIGH B-GALACTOSIDASE ACTIVITY

S.H. Son[1], H.L. Jeon[1], S.J. Yang[1], N.K. Lee[1], H.D. Paik[1]
[1]Konkuk University, Department of Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea

Backgrounds

Kimchi is a Korean traditional fermented vegetable dish, one of the healthiest foods in the world. Kimchi contains many lactic acid bacteria producing enzymes that can help lactose intolerant patients. β-Galactosidase, one of useful enzymes hydrolyzes lactose to galactose and glucose.

Objectives

The aim of this study was to isolate potential probiotic strains from kimchi that show probiotic properties and high β-galctosidase activity and reduce lactose content of yogurt.

Methods

A potential probiotic strain was isolated from kimchi using *Lactobacillus* selective agar. Among isolates from kimchi, *Lactobacillus plantarum* Ln4 had high acid tolerance (pH 2.5 with 0.3% pepsin, 3 h) and bile salt tolerance (0.3% oxgall, 24 h). *L*. *plantarum* Ln4 produced a useful enzyme according to the API zym kit and showed high capacity for adhesion to human colon adenocarcinoma cell line HT-29. *L*. *plantarum* Ln4 was sensitive to a commercial antibiotic according to a paper disc diffusion method. β-Galactosidase activity of *L*. *plantarum* Ln4 was evaluated using ortho-nitrophenyl-β-galactosidase (ONPG) and the result was 3,320.99 Miller units. Probiotic yogurt containing *L*. *plantarum* Ln4 showed reduced lactose contents as compared with commercial yogurt.

Conclusions

Therefore, *L*. *plantarum* Ln4 isolated from kimchi has probiotic properties and high β-galactosidase activity and can be used as a probiotic starter culture in the dairy industry.

FEMS7-0788
Food Microbiology - Part III

AN ANTIDIABETIC EFFECT OF NOVEL PROBIOTIC LACTOBACILLUS STRAINS ISOLATED FROM KIMCHI

S.H. Son[1], H.L. Jeon[1], S.J. Yang[1], N.K. Lee[1], H.D. Paik[1]
[1]Konkuk University, Department of Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea

Backgrounds

Kimchi is made of various vegetables such as cabbage, salt-fermented seafood, and red pepper, and is a Korean fermented food containing various lactic acid bacteria. Isolates from kimchi have probiotic characteristics and functional properties such as antioxidant and antidiabetic effects.

Objectives

The aim of the study was to screen *Lactobacillus* strains isolated from kimchi to identify those with probiotic properties and an antidiabetic effect.

Methods

Twenty *Lactoabacillus* strains were isolated from kimchi using *Lactobacillus* selective agar. Among the 20 *Lactobacillus* strains, 4 showed high resistance to acid (pH 2.5 with 0.3% pepsin, 3 h) and basic conditions (0.3% oxgall, 24 h). Enzymatic activities of 4 *Lactobacillus* strains were not indicative of a harmful enzyme according to the API zym kit. The 4 *Lactobacillus* strains had high capacity for adhesion to HT-29 cells and were sensitive to commercial antibiotics such as ampicillin, tetracycline, chloramphenicol, and doxycycline according to a paper disc diffusion method. The 4 *Lactobacillus* strains were identified as *Lactobacillus plantarum* Lb41, and *L. brevis* B151, G1, and KU15006. Among the 4 probiotic strains, a cell-free supernatant and intact cells of *L*. *brevis* G1 had the highest α-glucosidase-inhibitory activity: 24.14% and 11.18%, respectively. *L*. *brevis* KU15006 isolated from kimchi had a strong antidiabetic effect.

Conclusions

Therefore *L*. *brevis* KU15006 may help diabetes patients undergoing antidiabetic treatment.

FEMS7-1542
Food Microbiology - Part III

MOLECULAR TYPING OF LISTERIA MONOCYTOGENES ISOLATED FROM FOOD OF ANIMAL ORIGIN

M. Sosnowski[1], B. Lachtara[2], K. Wieczorek[1], J. Osek[1]
[1]National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Pulawy, Poland
[2]National Veterinary Research Institute, Department of Hygiene of Animal Origin, Pulawy, Poland

Backgrounds

Pulsed-Field Gel Electrophoresis (PFGE) subtyping analysis is one of the most important molecular method for the characterization of *Listeria monocytogenes*, an important epidemiologically foodborne pathogen.

Objectives

The aim of the study was genotypic characterization of *L. monocytogenes* isolated from food of animal origin in various part of Poland in years 2013 – 2016.

Methods

A total of 130 strains tested were classified into serogroups 1/2a, 1/2b, 1/2c and 4b, which are most commonly associated with human infections. The PFGE method with two digested enzyme *Asc*I and *Apa*I was applied to generate combined dendrograms and to assess a degree of genetic relatedness using 95% of similarity in different serogroups.

Conclusions

In each serogroup the following number of PFGE profiles were specified: 18, 16, 7 and 36 in 1/2a, 12/b, 1/2c and 4b serogroups, respectively. The highest diversity was observed in group 1/2b, where 11 profiles (68.8%) were represented by only one strain. The most genetic relatedness was demonstrated in 1/2c serogroup, where 30 out of 33 strains (90.1%) belonged to 4 pulsotypes with 3 to 17 strains. Most of the strains clustered into individual patterns were originated from different food products and were isolated in different part of Poland at various times.
A high genetic diversity among *L. monocytogenes* strains of 1/2a, 1/2b and 4b serogroups was showed. However, a significant degree of relatedness in 1/2c was observed.
Funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

FEMS7-1546
Food Microbiology - Part III

DISTRIBUTION OF LISTERIA MONOCYTOGENES SEROGRUPS AMONG DIFFERENT FOOD CATEGORIES AND ENVIRONMENTAL SAMPLES IN POLAND

K. Wieczorek[1], M. Sosnowski[1], B. Lachtara[1], J. Osek[1]
[1]National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Pulawy, Poland

Backgrounds

Listeriosis caused by *L. monocytogenes* is one of the most common food-borne illness with the high mortality rate. These ubiquitous bacteria may be found in various foods matrices: such as ready to eat food (RTE), fish and meat.

Objectives

The aim of this study was to assess the prevalence of four serogroups: IIa, IIb, IIIc and IVb, responsible for most cases of human listeriosis, among *L. monocytogenes* from various food and environmental samples.

Methods

A total of 596 *L. monocytogenes* strains were examined. The isolates were originated from 7 food categories: meat, fish, cheese, processed meat, delicatessen food, swab from processing plants and others. Multiplex PCR was performed to determine serogroups.

Conclusions

It was found that serogroup IIa was the most prevalent (n=280, 47.0%) followed by serogrup IVb (n=136, 22.8%), IIc (n=100, 16.8%) and IIb (n=80, 13.4%). Additionally, serogroup IIa dominated in fish (85.7%) and cheese products (84.6%). In remaining food categories incidence of this serogroup ranged from 42.1% (processed meat) to 55.0% (swab). On the other hand, the least marked serogroup IIb was not found in fish and in other categories was presented at the level from 4.3% (meat) to 37.5% (others).

This data gave evidence that serogrups of *L. monocytogenes* associated with human listeriosis may be present in wide range of food categories and suggest that this pathogen may represent a potential threat to public health.
Funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

FEMS7-1119
Food Microbiology - Part III

THE EFFECT OF REPLACING SYNTHETIC PRESERVATIVES WITH NATURAL ONES ON FRANKFURTERS' SHELF-LIFE

G. Stefan[1], C. Predescu[1], C. Papuc[1], I. Gajaila[1], V. Nicorescu[1], A.E. Rus[2]
[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, Bucharest, Romania
[2]Banat's University of Agricultural Sciences and Veterinary Medicine of Timisoara, Food Science, Timisoara, Romania

Backgrounds

Plant polyphenols and fermented juices rich in nitrite may be used as preservatives in meat industry.

Objectives

The aim of this study was to evaluate the effects of replacing ascorbate (AS) with hawthorn (*Crataegus monogyna*) polyphenols (HP) and synthetic nitrite (SN) with parsley (*Petroselinum crispum*) roots fermented juice nitrite (PRFJN), in different concentrations and relations, in unsmoked frankfurters.

Methods

The antioxidants and nitrite were used according to the following scheme: (1)50 ppm AS and 50 ppm PRFJN, (2)50 ppm HP and 50 ppm PRFJN, (3)0 ppm antioxidant and 50 ppm PRFJN, (4)25 ppm AS and 25 ppm PRFJN, (5)25 ppm HP and 25 ppm PRFJN, (6)0 ppm antioxidant and 25 ppm PRFJN, and (7)50 ppm AS and 50 ppm SN, as control. pH, thiobarbituric acid reactive substances (TBARS), cure efficiency, residual nitrite, total viable counts (TVC), *Enterobacteriaceae (EB)*, *Escherichia coli* and coliform bacteria (CB) were determined at 3-day intervals for 15 days. During storage, comparatively with (7), the best physico-chemical parameters were found for 1, 4, and 5 lots, and the best microbiological parameters for 1, 2, and 3 lots. For 1, 2, and 3 lots, the TVC, EB, *E. coli* and CB values were lower than the values found in the lots 4, 5, 6, and 7.

Conclusions

Replacement of AS with HP and SN with PRFJN was able to improve frankfurters shelf-life. This work was carried out through *Partnerships in priority areas* Program – PN II, implemented with the support of MEN – UEFISCDI (Romania), project nr. 149/2014.

FEMS7-2027
Food Microbiology - Part III

THE INFLUENCE OF DIFFERENT NITROGEN AND TEMPERATURE CONDITIONS ON MIXED CULTURE FERMENTATION BETWEEN S. UVARUM, S. EUBAYANUS STRAINS AND S. CEREVISIAE STRAIN

Y. Su[1], C. Lopes[2], A. Querol[1], J.M. Guillamón[1]
[1]Instituto de Agroquímica y Tecnología de los Alimentos, Departamento de Biotecnología de los Alimentos, Valencia, Spain
[2]Instituto de Investigación y Desarrollo en Ingeniería de Procesos, Biotecnología y Energías Alternativas, Buenos Aires, Argentina

Backgrounds

S. eubayanus and *S. uvarum* are two cryotolerant yeast species which are closely related with cider, beer and wine industry.

Objectives

We would like to explore phenotypically nitrogen requirement of different strains of *S. eubayanus* and *S. uvarum* species, and conduct competition fermentation with *S. cerevisiae*.

Methods

Nitrogen requirement was monitored by their growth and fermentation ability at different nitrogen concentrations. Subsequently, one low nitrogen demand strain of each species was chosen and competition fermentation was carried out with *S. cerevisiae* respectively under different nitrogen and temperature conditions.

Conclusions

In general, *S. eubayanus* strains used in our study have a lower nitrogen demand than *S. uvarum* strains. For the competition fermentation with *S. cerevisiae*, at low temperature (12°C), *S. cerevisiae* is not able to outweigh neither *S. eubayanus* nor *S. uvarum*, regardless of nitrogen concentration. On the contrary, *S. cerevisiae* completely out competed both non-*cerevisiae* species at high temperature fermentation (28°C), regardless of nitrogen concentration. At an intermediate temperature (20°C), in nitrogen limited condition, the maximum population of each species in the mixed culture was similar, however in non-limiting nitrogen condition, the percentage of *S. cerevisiae* in the mixed culture was higher than the other two species. Therefore, the competition experiments clearly evidenced that low temperature and low nitrogen concentration condition increase the competitiveness of the non-*cerevisiae* species. This result is of paramount importance for the industrial use of these alternative yeast species because of some traits of interest as lower ethanol yield and higher glycerol and aroma production.

FEMS7-2193
Food Microbiology - Part III

INTERCELLULAR PRODUCTION OF HYDROGEN PEROXIDE AS A PREVENTION TO SALMONELLA PROLIFERATION

G. Sutton[1], *M. Marvasi*[1]
[1]*Middlesex University, Natural Sciences, London, United Kingdom*

Backgrounds

Due to an increase in produce associated gastroenteritis, it is becoming clearer that we know little about the ecology of human pathogens in vegetables. Research has shown that *Salmonella* (14028) can grow in numbers up to 10^5 CFU/fruit compared with the initial inoculum. It was also found that Piccolo tomatoes were less conductive to *Salmonella* than larger cultivars.

Objectives

We hypothesise that cherry tomatoes naturally produce higher levels of H_2O_2 in their intercellular fluid than larger tomatoes.

Methods

Around 100 cells of *Salmonella* were inoculated in wounds of both Piccolo and Alicante cultivars. *Salmonella* proliferation was measured after a week of incubation by plating on Xylose lysine deoxycholate agar (XLD agar). To test H_2O_2 levels a novel method was used. Pieces of the pericarp (from both cultivars) totalling 2g were subjected to a pressured vacuum to infiltrate distilled water into the intercellular space. The pieces were then spun in a centrifuge to extract all fluid from the intercellular space. Samples were de-proteinised using 10kD spin columns. A fluorometric assay was used to test the concentration of H_2O_2. Multiple biological and technical replicas were carried out.

Conclusions

The increase of *Salmonella* from one week of initial incubation was up to 3.6 log,(CFU)/tomato in Alicante cultivar (SE=0.09), and 2.6 log,(CFU)/tomato in the cherry cultivar (SE=0.21). The difference in *Salmonella* proliferation was significant (α=0.05). The cherry tomatoes showed an average of 0.76nmol/ml of H_2O_2 whereas the Alicante cultivar showed an average of 0.46nmol/ml. The difference in H_2O_2 concentration between the tomato cultivars was significant. We speculate that *oxyR* gene in *Salmonella* activates the regulation of H_2O_2 inducible genes in tomatoes.

FEMS7-0643
Food Microbiology - Part III

IDENTIFICATION OF HEALTHY AND TECHNOLOGICALLY HAZARDOUS ASAIA SPP., ISOLATED FROM FOOD PRODUCTS AND PRODUCTION ENVIRONMENT, USING GENOTYPIC AND PHENOTYPIC METHODS

E. Svirakova[1], J. Kyznar[1], S. Purkrtova[2], R. Karpiskova[3], M. Jelinkova[4], J. Felsberg[4], I. Nemeckova[5]
[1]University of Chemistry and Technology Prague, Department of Food Preservation, Prague 6, Czech Republic
[2]University of Chemistry and Technology Prague, Department of Biochemistry and Microbiology, Prague 6, Czech Republic
[3]Veterinary Research Institute, Department of Bacteriology, Brno, Czech Republic
[4]The Czech Academy of Sciences, Institute of Microbiology of the CAS, Prague 4, Czech Republic
[5]Dairy Research Institute, Department of Technology and Microbiology, Prague 6, Czech Republic

Backgrounds

Bacteria from the *Asaia* genus represent technologically hazardous bacteria contaminating food products and processing environment due to worldwide globalization. *Asaia bogorensis* and *Asaia lannensis* are opportunistic pathogens causing serious nosocomial infection in immunocompromised individuals.

Objectives

The aim of this work is the identification and characterization of *Asaia* spp., isolated from food products and production environment, using genotypic and phenotypic methods.

Methods

The 16S rRNA gene sequencing and the MALDI-TOF MS methods were used for identification of tested *Asaia* spp. on the species level. The ERIC-PCR method was used for verification of its applicability for isolates. The optical microscopy method, the agar plate method, the densitometric method and the disk diffusion method were used for estimation of *Asaia* spp. characteristics.

Conclusions

Using the MALDI-TOF MS method, the database of main protein spectra for *Asaia krungthepensis* CCM 7333, *Asaia lannensis* BCC 15734 and *Asaia siamensis* CCM 7132 was created. All unknown isolates were identified as *Asaia lannensis* using the MALDI-TOF MS method at the highly probable species identification level. Likewise, these isolates were identified as *Asaia lannensis* using the 16S rRNA gene sequencing method; the consensus of obtained *Asaia lannensis* gene sequences with similar presented in available databases was minimally 99 %.The obtained results could be useful for effective assurance of health safety and quality of food products using modern identification methods providing quick, damning and reliable results. This work was supported by the Ministry of Agriculture of the Czech Republic, by the National Agency for Agriculture Research, by the project QK1710156 (2017−2021, MZE/QK).

FEMS7-0724
Food Microbiology - Part III

EARLY BLOWING IN RAW GOATS' MILK CHEESE: ENTEROBACTERIACEAE LEVELS AND EYES FORMATION DURING CHEESE RIPENING.

R. Tabla[1], A. Gómez[1], A. Simancas[2], I. Roa[1], F. Molina[2]
[1]CICYTEX-INTAEX, Dairy products, Badajoz, Spain
[2]Universidad de Extremadura, Bioquímica- Biología Molecular y Genética, Badajoz, Spain

Backgrounds

Enterobacteriaceae activity can influence cheese ripening; it may even be responsible for some specific characteristics of artisanal cheeses. Some specific characteristics of artisanal cheeses, such as higher proteolysis and lipolisis, have been correlated with *Enterobacteriaceae* activity. However, gas production capability of many *Enterobacteriaceae* can lead to early blowing in cheese, especially when initial population is high.

Objectives

The aim of this work was to study early blowing in raw goats' milk cheese, eye formation during ripening and its relationship with *Enterobacteriaceae* population.

Methods

Two different batches of cheese from raw goats' milk produced according to the PDO "Ibores" regulation (OJEU, 2004) were selected. One of them showed early blowing and the other was a typical one. Cheeses were cut in half at 7 and 60 days of ripening. The inner surface was digitalized using a densitometer (Bio–Rad GS800) and analysed using Fiji software. If present, mechanical holes were visually identified and erased. Additionally, *Enterobacteriaceae* levels and total mesophilic aerobic bateria were determined at 0, 7, 15, 30 and 60 days of ripening.

Conclusions

Although initial levels were similar for both batches, microbial growth was more intense in cheese with early blowing defect. Moreover, their ripening conditions were not able to inhibit *Enterobacteriaceae* growth. Most of the eyes in cheese were small sized and were formed during the first week of ripening. However, in defective cheese, big fissures appeared by the end of ripening.

FEMS7-2768
Food Microbiology - Part III

PHENOLIC ANTIOXIDANT MOBILIZATION IN BLACK GRAPE, APPLE AND DRAGON FRUIT RESIDUES BY ENZYMATIC TREATMENT USING A CELLULOLYTIC COCKTAIL FROM RHIZOMUCOR MIEHEI

C. Zambrano[1], F. Kovács[1], A. Kotogán[1], T. Papp[1,2], K.C. Mondal[3], F. Hargitai[1], C. Vágvölgyi[1], J. Krisch[4], M. Takó[1]
[1]University of Szeged, Department of Microbiology, Szeged, Hungary
[2]University of Szeged, HAS-USZ "Momentum" Fungal Pathogenicity Mechanisms Research Group, Szeged, Hungary
[3]Vidyasagar University, Department of Microbiology, Midnapore, India
[4]University of Szeged, Institute of Food Engineering, Szeged, Hungary

Backgrounds

There is growing interest for application of phenolic antioxidants as additives in functional foods. Therefore, the bioprocesses able to produce bioactive phenolics from natural sources have significant attention in the food industry. Many fruit residues contain such phytochemicals, but most of them are in glycosidic form having reduced bioavailability. However, it is known that the carbohydrate-cleaving enzymes can release polyphenols from their glycosides.

Objectives

In our previous experiments, a cellulolytic cocktail with high beta-glucosidase activity was produced from *Rhizomucor miehei* on wheat bran. Moreover, we proved that the beta-glucosidase of the fungus can increase the amounts of free phenolic antioxidants in sour cherry pomace. Here we applied this cellulase mix to enrich phenolic antioxidants from black grape, apple and dragon fruit residues.

Methods

Oven dried and lyophilized samples were mixed with *R. miehei* cellulase cocktail with or without addition of commercial *Aspergillus niger* pectinase. The reaction mixtures were incubated at 50°C for 5 or 24h under constant stirring, then, total phenolic content and antioxidant activity were analyzed in the clear supernatant.

Conclusions

Total phenolic content of the samples generally increased during the enzymatic treatment; however, its rate was different in each type of fruits, and depended on the drying method used for pomace pretreatment as well. In most samples, the antioxidant activity exhibited correlation with the total phenolic content. Addition of pectinase also supported the content of free antioxidative phenolics, which can be further improved by prolonging the incubation time to 24h. This research was supported by the NKFIH PD 112234 and GINOP-2.2.1-15-2016-00006.

FEMS7-0488
Food Microbiology - Part III

DETECTION AND IDENTIFICATION OF MYCOBACTERIA IN FOODS OF ANIMAL ORIGIN FROM SUPERMARKETS

M. Tello[1], E. Molina[1], N. Elguezabal[1], M. Serrano[1], R.A. Juste[2], J.M. Garrido[1], I.A. Sevilla[1]
[1]NEIKER - Tecnalia, Sanidad Animal, Derio, Spain
[2]SERIDA, Managing Director, Villaviciosa, Spain

Backgrounds

The genus *Mycobacterium* includes worldwide distributed obligate and opportunistic pathogens able to infect humans, livestock and wild animals. Mycobacteria are highly resistant to heat treatments and other adverse situations. Therefore, the consumption of contaminated livestock products could represent a route of mycobacteria transmission and pose a risk to human health.

Objectives

To study the exposure level to mycobacteria through the consumption of livestock products available in supermarkets using microbiological and molecular biology techniques.

Methods

Five supermarkets belonging to important chains were selected. 138 samples of dairy products and 119 samples of meat products were purchased at two different time points in order to analyse two independent batches of most of the products under study. All samples were submitted to several mycobacterial culture protocols and to a tetraplex (Real-time) PCR able to detect the genus *Mycobacterium*, *M*. *avium* and *M*. *tuberculosis* complex. Further identification was performed using additional molecular methods.

Conclusions

M. avium subsp. *hominissuis* (2), *M. avium* subsp. *avium* (1) and *M. fortuitum* (1) were isolated from one dairy (infant formula) and 3 meat products, respectively. Mycobacterial DNA (*M. avium*, *M. tuberculosis* complex and other mycobacteria) was detected in 15.22% of dairy products and 1.68% of meat products. Even if mycobacteria isolation and DNA detection was scattered, obtained results indicate that some contact with mycobacteria could occur through the consumption of these products. Thus, performing a thorough evaluation of the risk posed by the entry of these microbes into the food chain could be of great interest.

FEMS7-0934
Food Microbiology - Part III

CONJUGATED LINOLEIC ACID PRODUCTION OF PROPIONIC ACID BACTERIA ISOLATED FROM MIHALIC CHEESE

G. Tirpanci Sivri[1], *O. Oksuz*[1]
[1]*Namık Kemal University, Food Engineering Department, Tekirdağ, Turkey*

Backgrounds

Mihaliç Cheese, a traditional cheese of Turkey, has some characteristic properties because of Propionic Acid Bacteria (PAB). It is known that *Propionibacterium* spp. are capable of producing conjugated linoleic acid (CLA) which has attracted much attention since it has been associated with potential anti-carcinogenic, anti-adipogenic, anti-atherosclerotic and anti-diabetogenic activities.

Objectives

The aim of this study was to evaluate the CLA isomerization capacity of PAB isolated from a traditional cheese.

Methods

Propionibacterium spp. were isolated from Mihaliç cheese provided from 10 different local producers. Isolates have been identified by using Bruker Microflex MALDI-TOF-MS. *Propionibacterium freudenreichii* ssp. shermanii and *Propionibacterium freudenreichii* ssp. freudenreichii were dominant isolates. They were tested for their ability to produce CLA from free linoleic acid in skim-milk medium at 30 °C for 72 hours under anaerobic conditions.

Conclusions

CLA formation after incubation was determined by GC-MS. CLA isomers, c9,t11-C18:2 and t10,c12-C18:2 as abundant isomers, were also determined. The yield was calculated as the ratio of total CLA content and the amount of free linoleic acid. The production of CLA by Propionic acid bacteria offers a possible mechanism for some health-enhancing properties and provides new opportunities for the development of functional foods.

FEMS7-1009
Food Microbiology - Part III

DETERMINATION OF ANTIMICROBIAL ACTIVITIES OF HAWTHORN (CRATAEGUS MONOGYNA) FRUIT AND SEED IN METHANOL EXTRACT

D.D. Altan[1], T. Gumus[1], G. Tirpanci Sivri[1]
[1]Namık Kemal University, Food Engineering Department, Tekirdağ, Turkey

Backgrounds

Hawthorn belongs to the tribe *Crataegeae* and member of the *Rosaceae* family that native to the Mediterranean region, including north Africa, Europe and Asia. It is reported that 21 *Crataegus* species have been identified in Turkey. *Crataegus monogyna* is one of the most common species. *Crataegus* commonly is known as hawthorn and stated with different names, including *"yemişen, ekşi muşmula, aluç, kuş yemişi" in different regions of Turkey. Crataegus* species have been used since ancient times, traditionally. Scientific evidence has demonstrated that *Crataegus* species have high antioxidant activity due to the presence of different bioactive compounds, such as flavonoids, vitamin C, organic acids, minerals.

Objectives

The purpose of this study was to evaluate the antimicrobial activities of Hawthorn fruit and seeds extract in methanol.

Methods

In this study, antimicrobial activity against five bacterial species (*Escherichia coli* O157:H7 ATCC 33150, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 7644, *Salmonella* Enteretidis ATCC 13076, *Vibrio parahaelmolyticus* ATCC 17802) using the disc diffusion method of methanol extracts were determined.

Conclusions

Extracts of hawthorn fruit and seeds showed antimicrobial activity against tested reference bacteria with a different zone diameter (7.44-14.40 mm). Seed extracts exhibited the highest antimicrobial activity against *Listeria monocytogenes* (9.16 mm), while fruit extract exhibited strong antimicrobial activity against *Vibrio parahaelmolyticus* (14.40 mm). It was understood that hawthorn fruit and seeds extracts have had potential to be utilized as antimicrobial agent.

FEMS7-1578
Food Microbiology - Part III

EARLY TRANSCRIPTIONAL RESPONSE TO BIOTIC STRESS IN MIXED STARTER FERMENTATIONS INVOLVING SACCHAROMYCES CEREVISIAE AND TORULASPORA DELBRUECKII

J. Tronchoni[1], J.A. Curiel[1], P. Morales[1], R. Torres-Pérez[1], R. Gonzalez[1]
[1]ICVV CSIC-Universidad de La Rioja-Gobierno de La Rioja, Enologia, Logroño, Spain

Backgrounds

Advances in microbial wine biotechnology have led to the recent commercialization of several non-*Saccharomyces* starter cultures. These are intended to be used in either simultaneous or sequential inoculation with *Saccharomyces cerevisiae*.

Objectives

We analysed the transcriptional response to co-cultivation of *S. cerevisiae* and *Torulaspora delbrueckii*. The study is focused in the initial stages of wine fermentation with the goal of better understand the microbial interactions that can be stablished during wine fermentation with mixed-starters.

Methods

Fermentations were carried out in bioreactors using synthetic grape must to mimic industrial conditions. Experiments were carried out in triplicate for fermentation kinetics, CO_2 performance and RNAseq analysis.

Conclusions

Both species showed a clear response to the presence of each other, even though the portion of the genome showing altered transcriptional levels was relatively small. Changes in the transcription pattern suggested a stimulation of metabolic activity and growth, as a consequence of the presence of competitors in the same medium. The response of *S. cerevisiae* seems to take place earlier, as compared to *T. delbrueckii*. Enhanced glycolytic activity of the mixed culture was confirmed by the CO_2 production profile during these early stages of fermentation. Interestingly, *HSP12* expression appeared induced by co-cultivation for both of *S. cerevisiae* and *Torulaspora delbrueckii* in the two time points studied. This might be related with a recently described role of Hsp12 in intercellular communication in yeast. Expression of *S. cerevisiae PAU* genes was also stimulated in mixed cultures.

Acknowledgments: MINECO (AGL2015-63629-R,RTC-2014-2186-2); EU (7FP-IRSES-GA612441); (JdC- JA.Curiel).

FEMS7-0575
Food Microbiology - Part III

CONTROL OF BIOFILMS FORMED BY LISTERIA MONOCYTOGENES ISOLATED IN BRAZILIAN TILAPIA-PROCESSING FACILITIES USING ENVIRONMENTAL-FRIENDLY ALTERNATIVES

D. Vázquez-Sánchez[1], J. Antunes Galvão[1], A. Kato[1], E. Micotti Gloria[1], M. Oetterer[1]
[1]University Of Sao Paulo / Luiz de Queiroz College of Agriculture, Agri-Food Industry- Food and Nutrition, Piracicaba, Brazil

Backgrounds

New factors that can increase the prevalence of bacterial pathogens have been recently introduced by the massive production of tilapia filets in Brazil.

Objectives

The aims of this study were to assess the biofilm-forming ability of *Listeria monocytogenes* isolated in Brazilian tilapia-processing plants and determine their resistance to chemical disinfectants and environmental-friendly alternatives.

Methods

Biofilms of eight *L. monocytogenes* strains (including ATCC 15313) formed on polystyrene and stainless steel surfaces for 5, 24 and 48 h at 25°C were examined. The effectiveness of sodium hypochlorite, peracetic acid, and eleven essential oils (*Cordia verbenacea, Corymbia citriodora, Cymbopogon winterianus, Eucalyptus camaldulensis, Eucalyptus staigeriana, Eucalyptus urograndis, Lippia sidoides, Melaleuca alternifolia, Melaleuca leucadendron, Pimenta pseudochariophyllus, Thymus vulgaris*) were determined against planktonic cells and 24-h-old biofilms in terms of minimal inhibitory concentration and logarithmic reduction in the number of viable biofilm cells per square centimeter.

Conclusions

A positive correlation (with $P<0.01$) between the native surface of strains and their ability to form biofilms on polystyrene and stainless steel was found at each time. L2 and L8 strains showed the highest biofilm-forming ability in stainless steel and polystyrene, respectively. In contrast, ATCC 15313 had the lowest. Peracetic acid and *Lippia sidoides* oil were the most effective disinfectants against planktonic cells and biofilms formed by L2 and L8, followed by *Thymus vulgaris*, *Pimenta pseudochariophyllus* and sodium hypochlorite. Therefore, *Lippia sidoides* oil can be considered as an effective disinfectant to control the proliferation of *Listeria monocytogenes* in fish-processing facilities and an environmental-friendly alternative to reduce the current pollution caused by chemicals.

FEMS7-0578
Food Microbiology - Part III

ENVIRONMENTAL-FRIENDLY ALTERNATIVES TO CONTROL BIOFILMS OF COAGULASE-POSITIVE STAPHYLOCOCCUS AUREUS ISOLATED IN BRAZILIAN TILAPIA-PROCESSING FACILITIES

D. Vázquez-Sánchez[1], J. Antunes Galvão[1], M. Rodrigues Mazine[1], E. Micotti Gloria[1], M. Oetterer[1]
[1]University Of Sao Paulo / Luiz de Queiroz College of Agriculture, Agri-Food Industry- Food and Nutrition, Piracicaba, Brazil

Backgrounds

Mass production of tilapia filets in Brazil have included new factors that can enhance the prevalence of pathogens in tilapia-processing plants.

Objectives

This study aimed to determine the biofilm-forming ability of coagulase-positive *Staphylococcus aureus* isolated in Brazilian tilapia-processing plants and their resistance to chemical and environmental-friendly disinfectants.

Methods

Biofilms formed by ten strains (including ATCC 6538) on polystyrene and stainless steel were analyzed after 5, 24 and 48 h at 25°C. The efficacy of sodium hypochlorite, peracetic acid, and essential oils of *Cordia verbenacea, Corymbia citriodora, Cymbopogon winterianus, Eucalyptus camaldulensis, Eucalyptus staigeriana, Eucalyptus urograndis, Lippia sidoides, Melaleuca alternifolia, Melaleuca leucadendron, Pimenta pseudochariophyllus* and *Thymus vulgaris* were determined against planktonic cells and 24-h-old biofilms in terms of minimal inhibitory concentration and logarithmic reduction of viable biofilm cells per cm^2.

Conclusions

The native surface of strains and their ability to form biofilms on polystyrene and stainless steel was positively correlated (with $P<0.01$) at each time. S8 and S10 strains showed the highest biofilm-forming ability in stainless steel and polystyrene, respectively. ATCC 6538 had a similar biofilm formation in both surfaces, being intermediate in comparison with the other strains. *Lippia sidoides*, *Thymus vulgaris* and *Pimenta pseudochariophyllus* oils were significantly ($P<0.05$) more effective against planktonic cells of S8 and S10 than peracetic acid and sodium hypochlorite. Meanwhile, *Lippia sidoides*, *Thymus vulgaris* and peracetic acid showed a significantly ($P<0.05$) higher effectiveness against biofilms than *Pimenta pseudochariophyllus* and sodium hypochlorite. Therefore, *Lippia sidoides* and *Thymus vulgaris* oils represent an effective and environmental-friendly alternative to control the spread of *S. aureus* in fish-processing facilities.

FEMS7-1498
Food Microbiology - Part III

RISK ESTIMATES ACCORDING TO THE ORIGIN, FOOD MATRIX, SEROTYPE AND ANTIMICROBIAL RESISTANCE OF LISTERIA MONOCYTOGENES STRAINS ISOLATED IN SANTIAGO, CHILE.

M. Vidal[1], D. Rivera[2], L. Lapierre[3], R. Vidal[4]
[1]Secretaria Regional Ministerial de Salud Region Metropolitana, Laboratorio de Salud Pública Ambiental y Laboral, Santiago, Chile
[2]Universidad Andrés Bello, Escuela de Medicina Veterinaria, Santiago, Chile
[3]Universidad de Chile, Facultad de Ciencias Veterinarias y Pecuarias, Santiago, Chile
[4]Universidad de Chile, Facultad de Medicina, Santiago, Chile

Backgrounds

Listeria monocytogenes is a ubiquitous bacterium widely distributed in nature, being the etiological agent of listeriosis, usually caused by eating food contaminated or by direct contact with infected animals. Listeriosis has a high case-fatality rate in susceptible groups, such as pregnant women, children and the elderly. There are not previous reports of antimicrobial resistance in isolates from food in Chile.

Objectives

Determine the risk associated to food types, origin, serotype and antimicrobial resistance phenotype in *L. monocytogenes* strains.

Methods

The study includes 222 isolates of *L. monocytogenes*. 182 isolates from different food types and 40 from clinical specimens. The antimicrobials tested were: ciprofloxacin, gentamicin, sulfamethoxazole-trimethoprim, erythromycin, cephalothin, ampicillin, penicillin-G and tetracycline. The Kirby Bauer (KB) test to evaluate phenotypical antibiotic sensitivity was used. Minimum inhibitory concentration (MIC), "gold standard" quantitative test, also was performed. A chi-squared test to determine the association between antimicrobial resistance, food matrix, origin and serotype of *L. monocytogenes* strains, was applied.

Conclusions

45 out of 222 (20.3%) strains showed different antimicrobials resistance profile. Most of them showed resistance to a single drug (58%). Clustering analysis shows five groups, in which was possible to distinguish three resistance profiles-origin associations; (i) only livestock origin strains, (ii) includes strains of human origin and (iii) was a mixture of clinical and food strains. There was no single profile of multidrug resistance, whether associated to food type or specific serotypes. Our analysis only established relationships (p=0.05) between strains from food matrix and antimicrobial resistance, mainly raw meat and fish.

FEMS7-2545
Food Microbiology - Part III

THE EXPRESSION OF THE CHITINOLYTIC SYSTEM OF LISTERIA MONOCYTOGENES IS SUBJECT TO DIFFERENT REGULATION DEPENDING ON CARBON SOURCE UTILISATION INCLUDING CELLOBIOSE AND GLUCOSE

M. Villoria Recio[1], *M. Halberg Larsen*[1], *H. Ingmer*[1]
[1]*Food safety and zoonosis, Veterinary disease biology, Frederiksberg C- københavn, Denmark*

Backgrounds

Listeria monocytogenes is a food-borne pathogenic bacterium which can cause fatal infections. Outside the host, this bacterium inhabits terrestrial and marine environments where chitin polymer is abundantly found and it serves as carbon and nitrogen source to chitinolytic bacteria. In *L. monocytogenes*, the chitinolytic system comprises two chitinases, ChiA and ChiB and a lytic polysaccharide monooxygenase (LPMO10). The chitinases are expressed during growth in soil supporting their role in environmental survival. Despite the absence of chitin in mammalian hosts, the chitinolytic system is also important for infection. The regulation of chitinases is complex and includes several central regulators, namely PrfA, σB, agr and Hfq.

Objectives

The objective of our study is investigating the regulatory mechanisms and induction cues of the chitinolytic system in order to better understand its role in the different life modes of *Listeria*. Specifically, the role of different carbon sources on the expression of the chitinolytic system was studied and related to virulence.

Methods

The expression of *chiA*, *chiB*, *Lm*LPMO10 and *actA* was measured quantitatively by qRT-PCR upon addition of chitin, glucose and cellobiose to wildtype cells and mutants lacking the chitinases or *prfA*. Bacterial cells were grown in microtiter wells and growth was followed by OD measurements.

Conclusions

Chitin is a strong inducer of the *chiB* gene in a low-carbon content medium. Whilst glucose can only support *chiB* induction in stationary phase in a chemically defined media, this study reveals that the addition of cellobiose reverts this effect and gives a new insight on the regulation of the chitinases.

FEMS7-0881
Food Microbiology - Part III

MILKING MACHINE BIOFILMS: BACTERIAL COMMUNITY COMPOSITION AND PREVALENCE OF ANTIBIOTIC RESISTANCE

M. Weber[1], B. Goepfert[1], M. Savin[1], S. Tueffers[1], A. Lipski[1]
[1]Food and Nutrition Science, Food Microbiology and Hygienics, Bonn, Germany

Backgrounds

Biofilms on milking machines are a source of contamination of raw milk and its products. They can facilitate the transmission of mastitis pathogens within herds and the dissemination of antibiotic resistances.

Objectives

Due to the close contact of bacterial cells within a biofilm, we analyzed the bacterial community composition of biofilms and the relation between the density of bacterial populations and the abundance of antibiotic resistance genes to reveal the impact of biofilms on horizontal gene transfer.

Methods

Swab samples of different parts of the milking machine of a dairy farm were investigated by culture-dependent and -independent methods. Spots in the milking system with enhanced microbial colonization were identified by colony counting on selective and non-selective media. The fraction of antibiotic resistant cells was quantified on media containing different β-lactams and tetracycline. Direct DNA extracts were obtained from each swab sample and used to create clone libraries of 16S rRNA genes. Isolates as well as clone sequences were identified by 16S rRNA sequencing to assess the bacterial diversity and to identify dominating bacterial groups and antibiotic resistant isolates. The DNA extracts were screened for different groups of antibiotic resistance genes by RT-qPCR.

Conclusions

Different parts of the milking machine displayed high biofilm cell density. A high bacterial diversity, also of antibiotic resistant strains, was detected for several bacterial phyla. Different antibiotic resistance genes were quantified by RT-qPCR and correlated with bacterial densities at the sampling points.
The impact of bacterial cell density on the abundance of resistant cells and resistance genes was discussed.

FEMS7-3209
Food Microbiology - Part III

IMPACT OF PECTIC OLIGOSACCHARIDES FROM APPLE POMACE ON THE ADHESION OF LACTIC ACID BACTERIA, PATHOGENS AND FAECAL BACTERIA TO THE CACO-2 CELLS

A. Wilkowska[1], A. Nowak[2], I. Motyl[2]
[1]Institute of Fermentation Technology and Microbiology, Biotechnology and Food Sciences, Łódź, Poland
[2]Institute of Fermentation Technology and Microbiology,
Faculty of Biotechnology and Food Sciences- Lodz University of Technology, Łódź, Poland

Backgrounds

Pectin-derived oligosaccharides (POs) are promising candidates for new-generation prebiotics.

Objectives

The aim of the study was to examine the POs from apple pomace (AP), as potential source of prebiotics, in terms of the impact of its presence on the adhesion of lactic acid bacteria, faecal bacteria (isolated from the cow faeces) and selected pathogens to the intestinal epithelial cells.

Methods

The potential of AP as a raw material for POs production by means of enzymatic and mild acid hydrolysis was evaluated. Oligosaccharides with different degrees of polymerization (DP) were assessed using high performance anion exchange chromatography. Adhesion test for lactic acid bacteria, faecal bacteria and pathogenes was performed using Caco-2 cells.

Conclusions

The strongest stimulation of adhesion results were noted in the presence of P2 preparation. Its composition contained oligosaccharides with a DP of 1-10, but the content of oligosaccharides with DP 7-10 were twice higher than in the P1 preparation. Hydrolysate number 3 (P3) contained only oligosaccharides of DP 1-4. Our results indicate that the obtained POs oligosaccharides affects the adhesion of lactic acid bacteria, but the phenomenon strongly depends on the strain. Faecal bacteria and tested pathogens show much weaker adhesion to the intestinal cells in the presence of tested oligosaccharides. These results indicate that the apple pomace hydrolysates can be used as prebiotics for human and animals, because they stimulate bowel colonisation with lactic acid bacteria and inhibit the development of infections caused by pathogens.

This work was supported through Narodowe Centrum Badań i Rozwoju, grant GEKON (Gekon2/05/267284/15/2015).

FEMS7-3211
Food Microbiology - Part III

INFLUENCE OF SORBIC ACID AND SODIUM PYROSULFITE ON THE PRODUCTION OF VOLATILE PHENOLS BY BRETTANOMYCES/DEKKERA BRUXELLENSIS IN BLUEBERRY WINE

A. Wilkowska[1]
[1]*Institute of Fermentation Technology and Microbiology, Biotechnology and Food Sciences, Łódź, Poland*

Backgrounds

Brettanomyces/Dekkera bruxellensis is considered to be a major cause of wine spoilage due to the production of off-flavors such as vinyl- and ethylphenols, N-heterocyclic compounds, acetic acid and biogenic amines. 4-ethyl-phenol and 4-ethyl-guaiacol are the most abundant off-flavors produced by this yeast responsible for a sensory defect known as 'Brett character' associated with aromas of barnyard, wet animal and horse-sweat.

Objectives

The aim of this study was to evaluate the volatile phenols produced by *B. bruxellensis* under a range of growth-limiting conditions.

Methods

The yeasts were cultured in a blueberry wine containing different quantities of growth inhibitors such as ethanol, SO_2 (sodium pyrosulfite) and sorbic acid. The formation of 4-ethylphenol and 4-ethyl-guaiacol was periodically monitored by SPME-GC/MS. The wine color was analyzed using CR-5 Chroma Meter (Konica Minolta). Hue angle, Chroma and total color difference were calculated from Hunter L, a and b values.

Conclusions

Comparing samples supplemented with sorbic acid (100-500 mg/L), the additional use of 35 mg/L of total sulfur dioxide significantly reduced the synthesis of volatile phenols: by 2-57% in wine containing 5% (v/v) of ethanol, and by 3-67% in 12% (v/v) wine. A dose of 100 mg/L of sorbic acid in combination with 100 mg/L of sulfur dioxide exerted an inhibitory effect on the production of volatile phenols in undiluted blueberry must. The results of this study suggest possible ways of controlling *B. bruxellensis* in wineries.

FEMS7-1900
Food Microbiology - Part III

DEVELOPMENT OF SPECIES-SPECIFIC PRIMERS FOR THE RAPID DETECTION OF THE 1, 3-PENTADIENE DEBARYOMYCES HANSENII PRODUCERS STRAINS

P. Wrent[1], E.M. Rivas[2], E. Gil de Prado[2], M.J. Valderrama[2], J.M. Peinado[2], M.I. de Silóniz[2]
[1]Univeridad Complutense de Madrid, Microbiology III, Madrid, Spain
[2]Universidad Complutense de Madrid, Microbiology III, Madrid, Spain

Backgrounds

In order to prevent microbial spoilage, preservatives such as sorbic acid or its salts, are added to foods. It has been confirmed that some strains belonging to the *D. hansenii* species are capable of spoiling foods by decarboxilation of sorbate and subsequent production of 1, 3-pentadiene. The volatile 1,3 pentadiene compound, produces a petroleum-, hydrocarbon-like unpleasant off-odour. Single nucleotide polymorphism of *PAD1* and *FDC1* genes are essential for the decarboxylation of phenylacrilic acids in *S. cerevisae*. However, there is a lack of an affordable and rapid method for the detection of 1,3 pentadiene producer strains.

Objectives

To developed a specific PCR assay for the rapid detection of 1,3 pentadiene *Debaryomyces hansenii* producer strains based in a putative *FDC1* homologues region present in this yeast species.

Methods

About 100 strains, most of them belonging to *D. hansenii* species, were used in this work from different Type Culture Collections or isolated in our laboratory. Some of them from 1,3-pentadiene spoiled foods. For the primer design we used a putative *FDC1* homologue region present in *D. hansenii* whose sequence was obtained from NCBI.

Conclusions

For first time to our knowledge, we describe in this work the *D. hansenii DhFDC* region, a putatively homologue sequence of the *S. cerevisiae FCD1/YDR539W* gene. The *DhFDC* region presents an identity of 66% with the *FDC1* gen of *S. cerevisiae* and is located in close proximity (528bp) to *DhPAD* gen, but with an opposite reading frame. The DhFDC region was a good marker of D. hansenii 1,3 pentadiene producer strains.

FEMS7-1291
Food Microbiology - Part III

PROTON TRANSFER REACTION-TIME OF FLIGHT-MASS SPECTROMETRY CHARACTERIZATION OF VOLATILE ORGANIC COMPOUNDS ASSOCIATED WITH KEFIR AND KEFIR-LIKE CEREAL-BASED BEVERAGES

A. Yépez[1], A. Algarra Alarcón[2], P. Russo[3], F. Biasioli[2], R. Aznar[1,4], V. Capozzi[3], G. Spano[3]
[1]Universitat de València, Microbiology and Ecology, Burjassot- Valencia, Spain
[2]Research and Innovation Centre- Fondazione Edmund Mach,
Department of Food Quality and Nutrition, San Michele all'Adige, Italy
[3]University of Foggia, Department of the Science of Agriculture- Food and Environment, Foggia, Italy
[4]Instituto de Agroquímica y Tecnología de Alimentos IATA-CSIC, Biotechnology, Paterna- Valencia, Spain

Backgrounds

Kefir is a health-promoting beverage that is produced by milk fermentation with a consortium of bacteria and yeasts. The development of cereal-based products is of particular interest to elude milk intolerances of sensitive consumers. Among the various techniques for VOC analysis, Proton Transfer Reaction - Time of Flight - Mass Spectrometry (PTR-ToF-MS) offers an on-line determination during fermentation, with rapid and direct measurements that led to highly informative analytical output.

Objectives

On-line monitoring of VOC associated with the headspace i) of classical kefir and ii) of three kefir-like cereal-based beverages using PTR-ToF-MS.

Methods

A commercial PTR-ToF-MS 8000 apparatus from Ionicon Analytik GmbH (Innsbruck, Austria) was used to analyse the headspace of milk, barley, corn and oat beverages fermented with two commercial kefir starter cultures. The ionization conditions in the drift tube were 110 °C, 2.30 mbar and 550 V. The vials with fermenting matrices were sampled in an automated fashion using an autosampler. All data detected and recorded by the PTR-TOF-MS were processed and analysed using MATLAB and in-house developed scripts written in the R programming language.

Conclusions

To our knowledge, this is the first report on the study of VOC associated with the kefir headspace using PTR-ToF-MS, the on-line monitoring of VOC released during kefir fermentation and the detection of VOC associated with the headspaces of kefir-like cereal-based beverages. This information can be useful to better understanding the kefir fermentation process, to enhance sensorial quality of the products, and to select candidate VOC biomarkers for the selection of new starter cultures.

FEMS7-1477
Food Microbiology - Part III

EVALUATION OF SPOILAGE INDEX FOR ANIMAL FEED UNDER DIFFERENT STORAGE CONDITIONS

K. Yi[1], S. Cho[2], S. Kang[1], D.W. Kim[3], Y.J. Choi[1], C.D. Jeong[4], S.S. Lee[4], S.K. Kim[1]
[1]Konkuk University, Department of Animal Science and Technology, Seoul, Republic of Korea
[2]Chonbuk National University, Department of Animal Science, Jeonju, Republic of Korea
[3]RDA, National Institute of Animal Science, Wanju, Republic of Korea
[4]Sunchon National University, Department of Animal Science and Technology, Suncheon, Republic of Korea

Backgrounds

Prevention of spoilage in animal feed is crucial for animal and human health. However, there is little knowledge about spoilage index for feed. Spoilage of feed can be occurred within its shelf life depending on its storage conditions. Once feed is spoiled, various toxic compounds are generated and affect animal nutrition and performance with economic loss.

Objectives

Temperature, humidity and moisture contents are important environmental factors and they can influence spoilage of feed during storage. Occurrence of unexpected microbial growth and consequent production of organic acids can be recognized as indicators for progression of spoilage. The present study was conducted to evaluate relationships between those environmental factors and spoilage indicators.

Methods

Fractional factorial experimental design with three factors and their three levels was performed. For factors (levels), temperature (factor A; 23, 30, 37°C), humidity (factor B; <30, 55, >90%) and moisture content (factor C; 12, 24, 36%) were employed and 15 runs Box-Behnken design was constructed. Animal feed consisted of corn (29.4%), wheat bran (69.6%) and soybean meal (1.0%) was stored in designed storage conditions for two weeks. For analysis, microbial enumeration, water activity and organic acid production were performed. Response surface model between environmental factors and spoilage indicators was estimated to evaluate spoilage index.

Conclusions

In occurrence of unexpected microbial growth, significant linear and quadratic effects were found at moisture ($p<0.05$). Significant interaction between humidity and moisture was detected ($p<0.05$). It was found that humidity and moisture showed significant linear effect on water activity of feed ($p<0.05$). In organic acid production, significant linear effect of moisture on lactic and acetic acids was observed ($p<0.05$). Significant interaction between temperature and moisture was shownin lactic acid production ($p<0.05$).

FEMS7-1910
Food Microbiology - Part III

TRANSCRIPTOMIC ANALYSIS OF THE HEAT STRESS RESPONSE FOR A COMMERCIAL BAKER'S YEAST SACCAROMYCES CEREVISIAE

R. Yilmaz[1], V. Purutcuoglu Gazi[2], D. Varol[3]
[1]Hacettepe University, Food Engineering Department, Ankara, Turkey
[2]Middle East Technical University, Statistical Department, Ankara, Turkey
[3]Middle East Technical University, Department of Statistics, Ankara, Turkey

Backgrounds

Saccharomyces cerevisiae constantly faces changes even harsh environments such as high temperature threaten their survival, at least, prevents them from performing optimally. It is exposed to various stresses during its propagation and industrial application. We described the global gene expression profile responses to heat stress, including heat shock and temperature shift for the yeast from local company.

Objectives

The aim of this study is to explore the effects of heat stresses on global gene expression profiles and to identify the candidate genes for the heat stress response in *Saccharomyces cerevisiae* by using microarray technology.

Methods

These yeast cells were grown at 30°C to obtain initial cultures. These initial culture cells were transferred to 25°C for 6 hours and then 37°C for 1 hour in order to apply the heat shock treatment. The same strategy was performed for the application of temperature-shift treatment. Then the DNA microarray analysis was used to examine the mechanism of the heat stress response of the *S. cerevisiae*. Here, the data from all hybridizations and array normalization were analyzed using the GeneSpringGX 12.1 (Agilent) and the R 2.15.2 program language. In the analysis, all required statistical methods were performed comparatively. For the normalization step, among alternatives, the RMA results were used. To determine differentially expressed genes under heat stress treatments, the fold-change and the hypothesis testing approaches were executed under various cut-off values via different multiple testing procedures. Then up/down regulated probes were functionally categorized via the PAMSAM clustering.

Conclusions

The transcriptome changes under the heat shock and temperature-shift stress treatments show that the number of differentially up-regulated genes among the heat shock proteins and transcription factors changed significantly and the change in temperature is one of the important environmental conditions affecting propagation and industrial application of baker's yeast. This study statistically analyzes this affect via one-channel microarray data.

FEMS7-0427
Food Microbiology - Part III

OUTER MEMBRANE VESICLE TRANSLOCATES SALMONELLA PATHOGENICITY ISLAND PROTEINS IN SALMONELLA ENTERICA SEROVAR TYPHIMURIUM

S. Kim[1], S.Y. Hwang[1], S.I. Kim[1], H. Yoon[1]
[1]Ajou University, Department of Molecular Science Technology, Suwon, Republic of Korea

Backgrounds

Salmonella enterica serovar Typhimurium is a primary cause of enteric diseases and has acquired a variety of virulence factors during its evolution into a pathogen. Secreted virulence factors interact with commensal flora and host cells and enable *Salmonella* to survive and thrive in hostile environments. Outer membrane vesicles (OMVs) released from many Gram-negative bacteria function as a delivery vehicle for complex molecules, including virulence factors.

Objectives

In order to understand the roles of OMV in virulence regulation of *Salmonella*, a proteomic analysis was conducted on OMVs from *S. enterica* serovar Typhimurium ATCC 14028.

Methods

S. Typhimurium OMVs were harvested under two different conditions mimicking the infection environments and were subjected to LC-MS/MS analysis. The results of proteome analysis were evaluated using western-blot analysis and fluorescence microscopy.

Conclusions

Proteomic profiling on *Salmonella* OMVs revealed that multiple virulence effectors produced from *Salmonella* pathogenicity islands (SPIs) were associated with OMVs. SPI effectors have been known to be secreted by type 3 secretion systems (T3SSs). OMV-mediated secretion of SPI proteins was verified using western blotting in a mutant lacking SPI-1/SPI-2 T3SSs and flagella system. OMVs possessing SPI-1 effectors, when treated to epithelial cells, increased the amount of F-actin contents in the host cell membrane. Considering the role of OMVs as a long distance delivery vehicle without bacterial interactions with host cells, secretion of effectors via OMVs shed light on a new virulence strategy in *Salmonella* infection. This study was supported by a grant 14162MFD972 from Ministry of Food and Drug Safety in 2017.

FEMS7-0783
Food Microbiology - Part III

RESPONSE SURFACE METHODOLOGY FOR ANTIBACTERIAL EFFECTS OF 3 NATURAL PRESERVATIVES AGAINST LISTERIA MONOCYTOGENES ON FRESH LETTUCE

H.H. Yu[1], M.W. Song[1], N.K. Lee[1], H.D. Paik[1]
[1]Konkuk University, Department of Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea

Backgrounds

Listeria monocytogenes is one of significant foodborne pathogens widely distributed in foods including vegetables. Therefore, the fresh-cut vegetable industry is searching for a preservative that kills food-poisoning bacteria and is safe for humans.

Objectives

The objective of this study was to develop a 3-dimensional response surface model for inactivation of *L. monocytogenes* on fresh lettuce by means of 3 natural preservatives, grapefruit seed extract (GSE), cinnamaldehyde (CA), and nisin at 4°C.

Methods

Following a central composite design, lettuce was inoculated with a cocktail of 3 strains of *L. monocytogenes* (ATCC 15313, H7962 serotype 4, and Scott A NADC 2045 4b) and treated with an antibacterial solution including GSE (0.64 to 7.36 ppm), CA (1.6 to 18.4 ppm), and nisin (0.48 to 5.5 ppm). Lettuce was stored at 4°C for 12 h. Surviving *L. monocytogenes* cells were enumerated on Oxford listeria selective agar after incubation at 37°C for 24 h.

Conclusions

Nisin alone reduced *L. monocytogenes* by 2 $\log(N/N_0)$. GSE and CA alone showed a low antibacterial effect that reduced *L. monocytogenes* by ~1 $\log(N/N_0)$. Nevertheless, while keeping CA at 10 ppm, nisin 6 ppm and GSE 8 ppm reduced *L. monocytogenes* by approximately 4 $\log(N/N_0)$. While keeping GSE at 4 ppm, nisin 6 ppm and CA 20 ppm reduced *L. monocytogenes* by approximately 4 $\log(N/N_0)$ on lettuce. Therefore, the combination GSE 5–6 ppm, CA 15–20 ppm, and nisin 5.5–6 ppm is an effective antibacterial solution against *L. monocytogenes* on fresh lettuce.

FEMS7-0795
Food Microbiology - Part III

OPTIMIZATION OF THE ANTIBACTERIAL EFFECTS OF GRAPEFRUIT SEED EXTRACT, CINNAMALDEHYDE AND NISIN AGAINST SALMONELLA SPP. ON FRESH LETTUCE

H.H. Yu[1], M.W. Song[1], N.K. Lee[1], H.D. Paik[1]
[1]Konkuk University, Department of Food Science and Biotechnology of Animal Resources, Seoul, Republic of Korea

Backgrounds

Contamination with *Salmonella* spp. has often been implicated in foodborne illness outbreaks associated with consumption of fruits and vegetables including apples, tomatoes, lettuce, and parsley. Therefore, manufacturers of fresh-cut vegetables have become interested in developing a food preservative that can reduce pathogens such as *Salmonella* spp. in fresh produce and is safe for humans.

Objectives

The aim of this study was to evaluate the effects of 3 natural preservatives, grapefruit seed extract (GSE), cinnamaldehyde (CA), and nisin, against *Salmonella* spp. on fresh lettuce using the response surface methodology.

Methods

Following a central composite design, lettuce was inoculated with a cocktail of *S*. Typhimurium and *S*. Enteritidis and a combined antibacterial solution including GSE (0.049 to 0.301 mg/ml), CA (0.032 to 0.368 mg/ml), and nisin (0.016 to 0.184 mg/ml). The lettuce was stored at 4°C for 24 h. Surviving *Salmonella* spp. cells were enumerated on Salmonella-Shigella (SS) agar after incubation at 37°C for 24 h.

Conclusions

In this model, while keeping nisin constant at 0.1 mg/ml, GSE and CA reduced *Salmonella* spp. counts by approximately 2 $\log(N/N_0)$. Nisin was not effective against gram-negative bacteria. Nevertheless, while keeping CA constant at 0.2 mg/ml, GSE and nisin reduced *Salmonella* spp. counts by approximately 2 $\log(N/N_0)$. Therefore, the combination GSE 0.25–0.3 mg/ml, CA 0.25–0.35 mg/ml, and nisin 0.1–0.15 mg/ml is an effective antibacterial solution against *Salmonella* spp. on fresh lettuce.

FEMS7-0772
Food Microbiology - Part III

METAGENOMIC ANALYSIS REVEALS SEASONAL FEATURES OF WILD LETTUCE IN SOUTH KOREA

Y.C. Yu[1], S.J. Yum[1], D.Y. Jeon[1], H.G. Jeong[1]
[1]Chungnam national university, Food Science and Technology, Daejeon, Republic of Korea

Backgrounds

Lettuce(*Lactuca sativa*) is the most popular vegetables in the world and also largely consumed in South Korea. Pathogens in microbiota of lettuce can be more harmful because lettuce is commonly eaten without cooking.

Objectives

This study was conducted to reveal the microbiota of wild lettuce in South Korea and compare the difference of the seasonal microbiota having potential pathogens.

Methods

We collected lettuces(n=30) from five different sites in South Korea in April and July. 16S universal primer that was constructed to avoid chloroplast's 16S rRNA gene was used to amplify the 16S rRNA gene that targeted on V5-V6 region. Amplicon sequencing was performed using the Illumina Miseq™ platform under manufacturer's instructions.

Conclusions

After trimming and qualifying of raw sequences, the average 74,543 reads were analyzed per each sample. In April group, Proteobacteria was the predominant phylum, however, Fimicutes was more abundant in July. Among dominant genera (a proportion over 5% at least one sample), *Exiguobacterium* was the dominant genus in both April and July. *Pantoea* known as including potential pathogenic species was the most dominant in April, whereas *Enterobacter* was second dominant genus in July. In most of the sites, there are potential pathogenic genera such as *Bacillus*, *Staphylococcus*, including pathogenic species. In a PCoA result, there are compartmented clusters between April and July. In order to confirm the potential pathogens in microbiota of wild lettuce, further study using more samples collected in various location is necessary.

FEMS7-2415
Food Microbiology - Part III

PREVALENCE AND VIRULENCE MARKERS OF YERSINIA ENTEROCOLITICA IN MEAT

A. Zadernowska[1], W. Chajecka-Wierzchowska[1]
[1]University of Warmia and Mazury in Olsztyn, Chair of Industrial and Food Microbiology, Olsztyn, Poland

Backgrounds

According to annual reports of the European Food Safety Authority, *Yersinia enterocolitica* is third most common enteropathogen responsible for food poisoning. Meat is regarded as the main source of these infections.

Objectives

The aim of the study was to analyse the incidence of *Y. enterocolitica* in raw meat and to determine the incidence of selected factors of virulence of the bacteria. A total of 254 samples were obtained from several local markets in Olsztyn, Poland. Samples were consisted in poultry meat, beef meat, pork meat, minced pork and beef meat, minced poultry meat.

Methods

All the samples were kept at 4°C during transport and during storage before analysis. The analysis was done according to PN-EN ISO 10273:2003 within 4 hours after purchase. Genomic DNA isolation was performed with the Genomic Mini kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions. Triplex PCR was carried out to amplify three *Y. enterocolitica* genes: *ail, ystA* and *ystB*.

Conclusions

Yersinia enterocolitica were found in poultry meat (2), pork (3) and minced pork and beef (2). All of the strains of *Y. enterocolitica* were classified as biotype 1A, none of them had the gene *yst*A responsible for the production of enterotoxin YstA or the gene *ai*l responsible for the production of adhesin Ail. The gene *yst*B, responsible for the production of enterotoxin Ystb, was found in 5 out of 7 strains.

FEMS7-2474
Food Microbiology - Part III

LAMP AND RAPIDCHEK SELECT FOR THE RAPID AND SENSITIVE DETECTION OF SALMONELLA SPP. IN MEAT

A. Zadernowska[1], *W. Chajecka-Wierzchowska*[1]
[1]*University of Warmia and Mazury in Olsztyn, Chair of Industrial and Food Microbiology, Olsztyn, Poland*

Backgrounds

Meeting the food safety standards and complying with risk management in the food chain makes it necessary to develop new, rapid and reliable methods for identification of microbiological hazards. LAMP (Loop-mediated isothermal amplification), developed by Notomi *et al.* in 2000, can specifically, sensitively and rapidly amplify nucleic acids utilizing a DNA polymerase enzyme with high strand displacement activity and two pairs of primers recognizing six independent sequences of a target gene under isothermal conditions. RapidChek Select is immunochemical-based method with application of phage as a selective agent for Salmonella. Phage attack cross reactive bacteria preventing them from causing a false positive reaction in the assay.

Objectives

The objective of the studies was to investigate the potential for detection of *Salmonella* in chicken meat with alternative methods: LAMP (Loop-Mediated Isothermal Amplification) and RapidChek Select (immunoassay method based on the lateral flow technology) and to compare the results with the findings obtained with the reference method ISO 6579.

Methods

Meat samples were purchased from a retail market in Olsztyn (northeast Poland). Portions of meat (25g) were artificially contaminated with *Salmonella* and/or another *Enterobacteriaceae* bacteria cells on three levels and were tested by three methods.

Conclusions

The sensitivity of the alternative method was 100% and the specificity was 100%. Both the application of the LAMP and RapidChek Select tests allow for a substantial reduction of waiting time for the results, which is particularly important for analyses of raw materials and products with a short shelf-life.

FEMS7-1358
Food Microbiology - Part III

AFLP CHARACTERIZATION OF MICROORGANISMS FOR A RAPID AND ACCURATE IDENTIFICATION IN WINE AND OTHER FOOD INDUSTRIES

D. García Martínez[1], O. Zafra[1], J. Calzada[1], E. Pérez Lago[1], R. Francisco[1], I. Baselga[1], C. Santos[1]
[1]Universidad Francisco de Vitoria, Experimental Sciences, Pozuelo de Alarcón Madrid, Spain

Backgrounds

Winemaking is a natural fermentation process driven by microorganisms present in the grape skin and in the environment. Nowadays, there is a great interest in the study of microorganisms involved in fermentation due to the influence they have in organoleptic properties. Our group carried out the analysis, by the AFLP technique, of 1200 yeasts randomly isolated from 17 vineyards to create a database that includes the species and strain specific alleles amplified with two selected pairs of primers. In this study, yeasts were isolated from musts that belong to Ribera de Duero PDO in Spain.

Objectives

Currently our group tends to improve the database and our efforts are focused in samples isolated of different PDO in order to contrast yeast, grapes, *terroir*…

Methods

We have chosen La Mancha PDO and musts from different white grape strains have been analyzed. The isolated yeasts have been characterized by ITS-RFLP and AFLP analysis. Fermentation experiments are in progress with selected strains with the aim to analyze the produced aromatic compounds and hence link microbiology with flavor.
Also, we are working in setting up the method to differentiate edible mushroom strains in order to keep a record and provide genetic tools that warrant integrity of strains

Conclusions

AFLP analysis has been proved to be an accurate method to differentiate wild yeasts allowing subsequent characterization of specific strains in fermentation processes in order to determine their oenological properties. The technique is now being tested in edible mushrooms in which the setting up is different and results are preliminary

FEMS7-0866
Food Microbiology - Part III

BACTERIAL DIVERSITY OF HIGH BACTERIAL COUNT RAW COWS´ MILK FROM THE BULK TANK OF DIFFERENT DAIRY FARMS IN GERMANY

J. Zimmermann[1], *A. Lipski*[1]
[1]*Institute of Nutrition and Food Science, Food Microbiology and Hygiene, Bonn, Germany*

Backgrounds

The bacterial load of raw milk directly affects quality and shelf life of the processed milk and dairy products. Psychrotrophic bacteria pose a serious problem because they are able to grow rapidly under cold storage and contribute to spoilage by proteolytic or lipolytic activity.

Objectives

We characterized the dominating contaminants from 38 raw milk samples with high microbial load (>100.000 cfu/mL) from different dairy farms and determined their diversity.

Methods

Total bacterial counts were determined as colony forming units at 30°C or 10°C. Numbers and percentages of colonies with different morphologies were determined, in order to calculate the bacterial diversity and to isolate and identify dominating species. Additionally, diversity was determined by culture-independent analyses of 16S rRNA sequences from DNA extracts. Bacterial diversity of raw milk samples was calculated with Shannon-Weaver and Shannon-Evenness indices.

Conclusions

Results of the culture-dependent and –independent approach revealed two different groups of raw milk samples: Samples with low diversity and dominating bacterial taxa and samples with higher diversity and without dominating bacterial taxa. The dominating taxa of the first group were identified as Gammaproteobacteria and Firmicutes, respectively. The psychrophilic Gammaproteobacteria showed spoilage potential by lipolytic or proteolytic activity at 10°C and/or 4°C and were able to grow rapidly under cold conditions. The mesophilic species of the phylum Firmicutes were identified as potentially pathogenic bacteria, known to cause mastitis in dairy cows. In this study, we demonstrated that both groups, psychrophilic and mesophilic bacteria, are able to represent the dominating contaminant in raw cows´ milk from bulk tanks.

FEMS7-3010
Free Subjects / Other

DETECTION OF INTESTINAL PARASITES WITH CONVENTIONAL AND MOLECULAR METHODS IN FOLLOW-UP HIV/AIDS CASES

Ö. Akgül[1], B. Sapmaz[2], Ö.F. Karasakal[1], H. Kırkoyun Uysal[3], Y.A. Öner[3]
[1]Üsküdar University, Vocational School of Health Services, İstanbul, Turkey
[2]Nisantasi University, Vocational School, Istanbul, Turkey
[3]Istanbul University Istanbul Medical Faculty, Medical Microbiology, Istanbul, Turkey

Backgrounds

In people living with HIV, several complaints related to the gastrointestinal system, mainly diarrhea can be determined.

Objectives

We tried to examine the existence of *Giardia* spp., *Blastocystis* spp., *Entamoeba histolytica*, *Dientamoeba* spp. and *Cryptosporidium* spp. in stool samples.

Methods

In our study, we aimed to detect the existence of intestinal parasites with conventional methods based on microscopy and with molecular methods based on multiplex-PCR in 90 ART naive or ART adherent HIV/AIDS cases.

Conclusions

An overall prevalence of 36.7% at least one intestinal parasitic infections was recorded and the prevalence of this infection was due to *Blastocystis* spp. (22.2%), followed by *Dientamoeba* spp. (13.3%), *Entamoeba histolytica* (4.4%), *Cryptosporidium* spp. (3.3%), *Giardia* spp. (2.2%) and multiple parasitic infections (7.7%). The type of sexual behaviors (especially homosexual intercourse) are related with the detection of intestinal parasites as statistically highly significant ($p<0.001$). The increase in CD4+ T lymphocyte counts was negatively associated ($p=0.062$) and the increase in viral load levels was positively associated ($p<0.001$) with intestinal parasite detection rates. A statistically significant difference was observed in the overall parasitic infection rate was higher in pre-ART participants than in on-ART participants ($p=0.002$) and was higher in participants with diarrhea than in on participants without diarrhea ($p=0.019$). Further studies using larger participants and based on the detection of more pathogens are needed to provide more insight into the true etiology of gastrointestinal complaints in HIV/AIDS cases.

FEMS7-2409
Free Subjects / Other

ANTIMICROBIAL ACTIVITY OF SIX PLANTS SEEDS EXTRACTS ON URINARY PATHOGENIC BACTERIAL STRAIN

D. AL-Kurjiya[1]
[1]*research institute of the university of Bucharest-ICUB -faculty of biology, microbiology, bucharest, Romania*

Backgrounds

The purpose of this study was to investigate the antimicrobial activity of different plant extracts against urinary pathogenic strains.

Objectives

to examination the ability of vegetal extracts as antimicrobial activity on gram negative bacteria.

Methods

The urine samples were taken from pregnant women with different ages, who were suffering from urinary tract infections. A number of nine urinary pathogenic strains (3 *Escherichia coli*, 3 *Klebsiella spp*., 2 *Proteus spp.* and 1 *P. aeruginosa)* after isolation and identification depending upon cultural characteristics and biochemical tests results. The aqueous and alcoholic seeds extracts of sweet seeds, honey melon, cucumber, celery, sweet violet, cinnamon, with the following concentrations: 1%, 5% and 10% were tested. Bacterial suspensions of 0.5 MacFarland density were prepared and seeded on Muller Hinton media, followed by the distribution of filter paper discs previously saturated with watery and alcoholic plant extracts and dried at room temperature for 1 hour. The plates were incubated at 37C^{o} for 24 hours. Then the diameters of the growth inhibition zones were measured and average values were calculated when more than one strain of the same species was used.

Conclusions

This study demonstrates that the investigated seeds extracts could represent a viable alternative for the treatment of urinary tract infections, particularly taking into account the increasing incidence of antibiotic resistance among the etiological agents of these most frequent infectious diseases. Their antimicrobial efficiency in both aqueous and alcoholic extract form demonstrate the richness and active principles and the potential for multi-pharmacological actions, correlated with reduced side effects.

FEMS7-2477
Free Subjects / Other

CELL ASSOCIATED AND SOLUBLE VIRULENCE FACTORS IN NON ALBICANS CANDIDA STRAINS ISOLATED FROM IMMUNOCOMPROMISED PATIENTS

D. AL-Kurjiya[1]
[1]*research institute of university of Bucharest -faculty of biology, microbiology, bucharest, Romania*

Backgrounds

to study the virulance factor of non- *albicans candida which are considered the main impotent reasons which responsible for pathogenicity*

Objectives

the purpose of this study is to establish the virulence phenotype of non -*albicans candida spp*. isolated from localized and systemic infections in immunocompromised patients.

Methods

a total of 37 non candida albicans spp strains i.e.,*C.glabrata*(10),*C.tropicalis* (7),*C.krusei*(6),*C.famata*(4),*C.parapsilosis* (4),*C.kefyr*(3)and *C.guilliermondi*(2)were isolated from various clinical specimens.the production of extracellular hydroponic enzymes was determined by using specific growth media i.e., gelatin agar(bovine serum albumin)(BSA) for protease (Prz) ,egg yolk agar for phospholipases(Pz) and blood agar for haemolysis(Hz),(the lowest the value ,the highest is the production of the respective virulence factor).the results were recorded semi quantitatively and expressed as ratio of the colony diameter to the diameter of the colony plus the precipitation/haemolysis zone .the fungal adherence to HeLa cells was assessed by Cravioto's adapted method. adherence indexes and pattern were established by microscopic evaluation .the biofilm formation was quantified by a modified violet crystal microtiter method,after 48h of incubation at 35Cº in Sabouraud dextrose broth supplement with 8%glucose,distributed in 96 multi well plates .the Candida albicans ATCC 10231 was used as reference.

Conclusions

the in vitro study of the virulence factors profiles in non -albicans candida spp strains isolated from different clinical infections is imperative for the understanding of their pathogenesis and correlation with certain infectious processes,aspect that could contribute to the proper the therapeutic management of fungal infectious .

FEMS7-2533
Free Subjects / Other

INVESTIGATION OF ANTIMICROBIAL EFFECT OF EXTRACTS MAHONIA AQUIFOLIUM

A. Damjanović[1], J. Antic Stankovic[2], S. Mijatovic[2], G. Zdunić[3], Z. Juranic[1]
[1]Institute of Oncology and Radiology of Serbia, Department for Experimental Oncology, Belgrade, Serbia
[2]Faculty of Pharmacy University of Belgrade, Departmet of Microbiology and Immunology, Belgrade, Serbia
[3]Department for Pharmaceutical Investigations and Development,
Institute for Medicinal Plant Research Dr. Josif Pančić, Belgrade, Serbia

Backgrounds

Mahonia aquifolium is one of the most abundant plants of the genus *Mahonia*, it belongs to the ornamental shrubs and is widespread in Europe and America. Preparations of this plant are used in traditional medicine, for the treatment of inflammatory conditions of the skin. It is believed that the berberine and the barberine type alkaloids are main holders of activity of the plants of this genus.

Objectives

The aim of the study was to investigate the effect of water and ethanol extracts of *M. aquifolium,* on the the survival of various ATCC strains of microorganisms (*S. aureus*, *S. mutans*, *B. subtilis*, *E. coli*, *K. pneumoniae*, *C. albicans*).

Methods

The effect of the extracts on the production of biofilm and glycocalyx, was determined after treatment of *S. aureus* with sub-inhibitory concentrations of extracts. Also, it was examined the influence of the extracts on the activity of antibiotics (amoxicillin, cephalexin, ceftriaxone, erythromycin, tetracycline, chloramphenicol, gentamicin, bacitracin) on the growth of *S. aureus* and antimycotics (nystatin and voriconazole) on the growth of *C. albicans*.

Conclusions

Obtained results showed that ethanol extract has better antimicrobial effect, in comparison with aqueous extract. Both extracts moderately reduce the production of virulence factors and show anadditive effect with tested antibiotics and antmycotics, on the tested strains of microorganism

FEMS7-2611
Free Subjects / Other

AUGMENTATION OF THE HYDROLYSIS OF DIETARY GLUCOSYLCERAMIDE IN THE LARGE INTESTINE BY A NEWLY BACTERIUM ISOLATED FROM CANINE FECES

N. Asanuma[1]
[1]*Meiji University, Life Science, Kawasaki, Japan*

Backgrounds

Ceramides and sphingoids are known to play various roles as second messengers in many aspects of cell regulation, including cell growth, cell differentiation, autophagy, and apoptosis. The major sphingolipids included in plant food are glucosylceramide (GluCer). However, dietary GluCer is barely hydrolyzed to ceramide in the intestinal tract, and most of GluCer is exerted in feces.

Objectives

Thus, we examined the method to enhance GluCer hydrolysis in the large intestine by intestinal bacteria.

Methods

A novel anaerobic bacterium having high ability to hydrolyze plant GluCer was isolated from canine feces, and designated *Bacterium_A*. To use this isolate as a probiotic, freeze-dried cells were administered to mice.

Conclusions

The 16S rRNA gene sequence analysis indicated that *Bacterium_A* was a member of the *Clostridium coccoides* rRNA group of organisms, but the divergence to other bacteria was more than 5 %. Oral administration of *Bacterium_A* cells to mice for 4 weeks had no effects on feed intake, body weight gain, appearance, or behavior. These results suggest that *Bacterium_A* is not acutely toxic to mice. When a low level of purified GluCer was fed to mice, some amount of GluCer was exerted in feces, indicating that even extracted or free GluCer is not completely hydrolyzed in the digestive tract. GluCer excretion by mice fed apple powder was similar to that with purified GluCer. Irrespective of the state of GluCer, administration of freeze-dried *Bacterium_A* cells significantly decreased the amount of GluCer recovered in feces, suggesting that GluCer is hydrolyzed by *Bacterium_A* mainly in the large intestine.

FEMS7-2356
Free Subjects / Other

ANTITUBERCULAR POTENCY OF 1, 3, 5, 6 AND 7 SUBSTITUTED 4-HYDROXYQUINOLIN-2(1H)-ONES

M. Bidart de Macedo[1], A. Peixoto[2], L. Verschaeve[3], P. Cos[1], G. Caljon[1], L. Maes[1], P. Delputte[1], J. Košmrlj[4], D. Cappoen[1]
[1]University of Antwerp, Department of Pharmaceutical Sciences, Wilrijk - Antwerp, Belgium
[2]Institut de Pharmacologie et de Biologie Structurale, Département Mécanismes Moléculaires des Infections Mycobactériennes, Toulouse, France
[3]University of Antwerp, Department of Biomedical Sciences, Wilrijk - Antwerp, Belgium
[4]University of Ljubljana, Department of Chemistry and Biochemistry, Ljubljana, Slovenia

Backgrounds

Approximately 2 billion people are infected with *Mycobacterium tuberculosis* and are hence at risk of developing active tuberculosis. There are several problems associated with the currently available treatment: nonadherence, potential side effects, and drug resistance. As a result, new treatments for tuberculosis are urgently needed. The search of newer antitubercular drugs led to the identification of several quinoline-based antituberculosis agents.

Objectives

The objective of this work was to search from the hydroxyquinolin-2(1*H*)-ones, a group of pharmacological active compounds with diverse antifungal and antibacterial activity, a candidate a new antituberculosis agent.

Methods

A library of 54-membered of substituted 4-hydroxyquinolin-2(1*H*)-ones and related compounds was designed, scored *in-silico* for drug likeness using the FAF-Drugs3 tool and subsequently synthesized. The minimal inhibitory concentration (MIC) was evaluated by serial dilution method, using a luminescent reporter strain *Mycobacterium tuberculosis* H37Ra^{lux} that harbors the pSMT1 plasmid carrying the *lux*AB genes from *Vibrio harveyi*. The *in vitro* cytotoxicity of the selected compounds was evaluated against MRC-5 human lung fibroblast cell and the early signs of genotoxicity were evaluated by Vitotox™ assay.

Conclusions

In this investigation, we have observed the antimycobacterial potency of a small library of 4-hydroxy-2(1*H*)-quinolones and structural related compounds. Derivatives carrying a 3-phenyl substituent were favored exhibiting low MIC and no signs of cytotoxicity or genotoxicity. Although the activity was not in the same order as the first line antituberculosis drugs, the library proved that the 4-hydroxy-2(1*H*)-quinolone can be altered by structural modification and change in substituents of the heterocyclic scaffold, representing, hence, an interesting group of antitubercular compounds.

FEMS7-0242
Free Subjects / Other

USE OF SACCHAROMYCES CEREVISIAE AND TRICODERMA VIRIDAE FOR LOW COST POULTRY FEED PRODUCTION IN BANGLADESH

A. Biswas[1], K. Kazi[2], H. Qurashi[3]
[1]Sunchon National University, Animal Science and Technology, Suncheon, Republic of Korea
[2]Bangladesh Agricultural Research Council, Krishi Gobesona Foundation, Dhaka, Bangladesh
[3]Chittagong Veterinary and Animal Sciences University, Animal Science and Nutrition, Chittagong, Bangladesh

Backgrounds

Low cost poultry feed ingredients like maize, wheat, rice polish, rubber seed contain low protein. Also, rice polish contain high fiber, so use of those ingredients in poultry ration is limited.

Objectives

This experiment was conducted to increase protein and vitamin level and decrease fiber level of locally available feed ingredients to formulate a low cost poultry feed.

Methods

Selected feed ingredients were mixed separately with *Saccharomyces cerevisiae* and *Tricoderma viridae* in a different combination. From fermented ingredient, samples were collected at 48 hours interval for crude protein, crude fiber estimation. Estimation of Vitamin A, D, K and toxicity level of fermented feed were done by UHPLC. On the basis of highest protein and lowest fiber containing ingredients, a low cost ration was formulated and an on-station also two on-farm trials on broiler were performed.

Conclusions

Crude protein content of these ingredient was increased from 9% to 12% for maize, 11% to 13% for rice polish, 11% to 14.5% for wheat and 17% to 20% for rubber seed. Treated rice polish was found to decrease crude fiber from 18% to 12%. Significantly higher carcass quality, feed conversion ratio (FCR) were ($p<0.01$) observed in treatments diet fed bird and no toxin was observed in fermented feed. In the on-station trial, cost of treatment feed was about 2.6 BDT/kg less compared to control feed and profit margin was 39.3 BDT/bird higher for treatment group bird compare to control. In case of on-farm trial, the average cost of treatment feed was 29.2 BDT/kg which was 1.8 BDT/kg less compared to control and profit margin was calculated to be 20.15 BDT/bird higher for treatment group bird compare to control.

FEMS7-2330
Free Subjects / Other

IMPROVING THE IN VITRO MODELS OF GASTROINTESTINAL DIGESTION: THE ROLE OF THE ORAL MICROBIOME

M. Calatayud[1], C. Xiong[2], K. Francesconi[2], G. Du Laing[3], T. Van de Wiele[1]
[1]Ghent University, Center for Microbial Ecology and Technology CMET, Ghent, Belgium
[2]University of Graz, Institute of Chemistry, Graz, Austria
[3]Ghent University, Department of Applied Analytical and Physical Chemistry, Ghent, Belgium

Backgrounds

The oral cavity contains a vast diversity of microorganisms. The alimentary bolus, composed by chewed food and saliva, reaches the stomach and small intestine, where different food components are digested and released for further absorption. Despite the relevance of the microbiome to the human physiology, little is known about the effect of microorganisms in the estimation of oral bioaccessibility (fraction of a specific compound released from its matrix during digestion) of drugs, toxicants or food compounds.

Objectives

Assess the effect of the oral microbiome into the *in vitro* bioaccessibility and intestinal transport of an environmental pollutant (arsenic).

Methods

An *in vitro* model with four stages (oral, gastric, small intestinal and colonic) was applied to rice, mussels and nori seaweed. Oral reactors were inoculated with salivary samples from healthy donors and colon reactors were inoculated with a fecal inoculum from the same donors. Oral reactors without the addition of saliva were run in parallel. After each digestion step, samples were centrifuged and filtered, and arsenic content was analyzed in supernatants. The supernatants from the small intestine and colon digestion were applied to a co-culture model of Caco-2 and HT29-MTX cells in Transwells® and arsenic transport and cellular uptake were assessed.

Conclusions

The presence of oral microbiota (saliva) in the *in vitro* model affected significantly the bioaccessibility of arsenic in a food-dependent way. The oral microorganisms increased the cellular uptake of the environmental pollutant in all the conditions. The current standardized *in vitro* methods for evaluating bioaccessibility are lacking a key element in the digestion process (oral and gut microorganisms), which could affect the *in vitro/in vivo* correlations. The combination of *in vitro* digestion models, including microbial metabolic potency, with models of the gut epithelium, can offer a more accurate prediction of As bioavailability, and in a broader context, environmental pollutants, drugs or food compounds.

FEMS7-0996
Free Subjects / Other

EXTENSIVE RECOMBINATION FOLLOWED BY ALMOST STRICT CLONALITY IN THE EMERGENCE OF THE MYCOBACTERIUM TUBERCULOSIS COMPLEX

A. Chiner-Oms[1], L. Sanchez-Busó[2], J. Corander[3], S. Gagneux[4], F. González-Candelas[1], S. Harris[2], I. Comas[5]
[1]FISABIO-Universitat de Valencia, Unidad Mixta Infección y Salud, Valencia, Spain
[2]Wellcome Trust Sanger Institute, Pathogen Genomics, Hinxton, United Kingdom
[3]University of Oslo, Department of Biostatistics, Oslo, Norway
[4]Swiss Tropical and Public Health Institute, Medical Parasitology and Infection Biology, Basel, Switzerland
[5]Biomedicine Institute of Valencia, Tuberculosis Genomics Unit, Valencia, Spain

Backgrounds

The role of recombination in the evolution of the *Mycobacterium tuberculosis* complex (MTBC) is debated. MTBC has been usually regarded as essentially clonal. The availability of hundreds of MTBC clinical strains as well as some of close relatives such as *M. canetti* provides the opportunity of analysing the microevolutionary events that led to the emergence of the tuberculosis bacillus.

Objectives

To analyse the role of recombination in the evolution of MTBC, firstly in shaping its current diversity, and secondly to characterise the ancestor of the MTBC just before its transition to the successful pathogen we know today.

Methods

Firstly, we checked for recombination events that occurred after the MTBC ancestor split from *M. canetti*. We analysed ~1600 representative strains of the global genetic diversity of the MTBC. All the analytical approaches used agreed in assigning a minimal role to recombination in the recent evolution of the MTBC. Also, when checking for recombination signal between *M. canetti* and MTBC strains no ongoing recombination was found.

Secondly, we searched for ancient recombination events. We found 67 recombination events between the branch leading to the MTBC ancestor and *M. canetti* branches. Genes in these regions were enriched for functions involved in symbiotic interactions inside a host cell.

Conclusions

From our results, recombination seems to have played a relevant role in the early speciation events and in the emergence of the MTBC. However, once the MTBC ancestor diverged into the current diversity, recombination has no longer been detected.

FEMS7-2905
Free Subjects / Other

CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE INFECTIONS: RISK FACTORS AND 30-DAY MORTALITY IN HOSPITALIZED PATIENTS FROM A KPC ENDEMIC REGION.

A.V. Cienfuegos[1], P. Sierra Viana[2], C. Restrepo Castro[2], F. Ramirez Brinez[2], G. Roncancio Villamil[3], H. del Corral Londoño[1], J.N. Jimenez Quiceno[1]
[1]Universidad de Antioquia, Antioquia, Medellin, Colombia
[2]IPS Universitaria Clínica León XIII, Antioquia, Medellín, Colombia
[3]Clínica CardioVID, Antioquia, Medellin, Colombia

Backgrounds

Carbapenem-resistant-*K.pneumoniae* (CRK) has become a worldwide threat due to its rapid spread and limited therapeutic options.

Objectives

The aim of this study is to identify risk factors and to determine 30-day-mortality in CRK infections.

Methods

A cohort enrolling all patients infected by *K.pneumoniae* in one hospital (700-beds) from Medellín-Colombia during 2014-2015 was analyzed. Clinical information was retrieved from charts. Risk factors were identified by a case-control analysis; cases were defined as patients infected by CRK and controls as patients infected by carbapenem-susceptible-*K.pneumoniae* (CSK). Analyses were done using logistic regression. To determine 30-day-mortality a survival analysis was performed. Models were fitted using a generalized-gamma and Weibull distributions because of non-proportionality of risks

Conclusions

In total 338 patients were enrolled, 49 (14.5%) infected by CRK and 289 (85.5%) infected by CSK. Most patients were male (58.5%) and adults (median=67-years IQR=51-76). Factors associated to CRK adjusted for time-at-risk were previous use of meropenem (OR=7.04, 95%CI=3.20-15.30), ciprofloxacin (OR=2.71, 95%CI=1.21-6.07) and urinary catheter (OR=2.35, 95%CI=1.16-4.77). Overall 30-day-mortality was 13.9% (n=47); 11 deaths occurred among CRK-infected-patients (22.5%) and 36 among CSK (12.5%)(p=0.062). Death incidence rate was 0.007 per person-days. In the bivariate analyses, age >67-years (HR=2.11, 95%CI=1.16-3.84) and Charlson index (HR=1.17, 95%CI=1.04-1.32), but not CRK-infection (HR=1.46 95%CI=0.74-2.88) were associate with higher mortality. In the multivariate analysis only Charlson index remained associated to 30-day-mortality. Previous use of carbapenems and ciprofloxacin in the last six months and urinary catheter play an important role in CRK-infection. Although death proportion was higher among CRK infected patients, comorbidities were the main risk for 30-day-mortality.

FEMS7-0706
Free Subjects / Other

PROBIOTIC POTENTIAL OF PREVALENT CULTURABLE LACTIC ACID BACTERIA ISOLATED FROM HUMAN MILK AT DIFFERENT STAGES OF LACTATION

M.H. Omura[1,2], E.C.C. Brumano[1,2], D.J. Silva[3,4], M.R. Eller[1,2], V.S. Duarte[2,5], L.R. Cunha[4,6], R.M.A. Araujo[2,7], C.L.L.F. Ferreira[1,2]
[1]Department of Food Technology
[2]Federal University of Viçosa, 36570 000 MG, Brazil
[3]Faculty of Medicine
[4]Federal University of Ouro Preto, MG, Brazil
[5]Department of microbiology
[6]Department of Food Engineering
[7]Department of Health and Nutrition

Background: Human milk (HM) is a complete food in terms of nutrition, immune precursors and microbial content, necessary to the newborn's (NB) development. Pasteurization is applied to HM deposited in the banks, eliminating most of the endogenous microorganisms.

Objective:To identify the prevalent culturable lactic acid bacteria (LAB) in colostrum, transition and mature HM and to verify their potential as probiotic.
Methods: Milk Samples from 14 nursing mothers were plated in Rogosa and modified MRS agar media. A total of 320 colonies were picked from the highest dilutions and coccus or bacillus, Gram positive, negative for gelatinase or beta hemolysis were evaluated for resistance to artificial gastric juice (AGJ), bile salts (BS), sensitivity to antibiotics (AS), growth in the presence of human blood serum (HBS) and antagonism to pathogens.

Results:The majority of strains were beta-hemolytic (297) and the final 23 isolates were identified as Enterococcus faecalis (E. faecalis) (11), Staphylococcus epidermidis (S. epidermidis) (9), S. lugdunensis (3). The species resisted well to the AGJ and to BS, antagonized indicator pathogens, grow moderately in the presence of HBS and the sensitivity to antibiotics was variable.

Conclusion: It is the first time that culturable LAB strains from HM were systematically isolated from mothers along their nursing period, E faecalis identifed as prevalent at different stages of lactation and probiotic characteristics evaluated. However the ecological function of the prevalence of such strains in the onset of the intestinal colonization of the NB deserves further investigation.

FEMS7-0690
Free Subjects / Other

SYNERGISTIC ACTIVITY OF MICAFUNGIN PLUS VORICONAZOLE AGAINST MULTIDRUG-RESISTANT CANDIDA AURIS: CANDIDEMIA

H. Fakhim[1], A. Chowdhary[2], J. Meis[3], V. Afsane[4], H. Badali[5]
[1]Mazandaran University of Medical Sciences, Department of Medical Mycology and Parasitology-, Tabriz, Iran
[2]University of Delhi,
Department of Medical Microbiology- Division of Mycology- Vallabhbhai Patel Chest Institute, Delhi, India
[3]- Canisius Wihelmina Hospital CWZ, Department of Medical Microbiology and Infectious Diseases, Nijmegen, Netherlands Antilles
[4]Mazandaran University of Medical Sciences, Department of Medical Mycology and Parasitology, Sari, Iran
[5]Mazandaran University of Medical Sciences- Sari- Iran,
Department of Medical Mycology and Parasitology, Sari, Iran

Backgrounds

Blood stream infections due to *Candida auris* is related to a high mortality rate, treatment failure, and resistant to fluconazole, voriconazole, amphotericin B, and caspofungin. Thus, the precise identification of agents and *in vitro* antifungal susceptibility testing is highly recommended. Novel therapeutic strategies, such as combination therapy, are essential for increasing the efficacy and reducing the toxicity of antifungal agents.

Objectives

We investigated the *in vitro* combination of micafungin plus voriconazole against multidrug-resistant *C. auris* as agents of candidemia.

Methods

A total of ten clinical isolates were obtained from tertiary care hospitals in Delhi, North India. Isolate identities were performed based on conventional methods and MALDI-TOF MS. The interactions of micafungin with voriconazole were investigated using a microdilution checkerboard technique in 96-well microtitre plates. The range of the concentration depended on the MIC results for each isolate; the maximum concentration was twofold the MIC and then serial diluted. Data obtained by visual reading were further analyzed using the fractional inhibitory concentration index (FICI).

Conclusions

Results revealed that MICs range for voriconazole and micafungin were 0.5-8 and 0.25–8 mg/L, respectively. The checkerboard analysis revealed that the combination of micafungin with voriconazole exhibited synergistic activity against all 10 multidrug-resistant *C. auris* isolates (FICI range: 0.15-0.5). Overall, no antagonistic effects were observed in this experiments.
Remarkably, the majority of isolates were resistant to fluconazole and unsuccessful treatment of *C. auris* infections with fluconazole, voriconazole, amphotericin B, caspofungin, and anidulafungin has been already on recorded. Further, interaction between micafungin with voriconazole exhibited synergistic activity against multidrug-resistant *C. auris* isolates. It seems that lower concentrations of drugs cause fewer side-effects and improve the treatment outcomes. However, *in vivo* studies with suitable animal models of *C. auris* infection is highly recomended.

FEMS7-0693
Free Subjects / Other

IN VIVO ANTIFUNGAL ACTIVITIES OF NOVEL AZOLE COMPOUNDS (ATTAF-1 AND ATTAF-2) IN A MURINE MODEL OF INVASIVE CANDIDIASIS

H. Fakhim[1], E. Dannaoui[2], A. Vaezi[3], S. Emami[4], H. Badali[5]
[1]Mazandaran University of Medical Sciences, Department of Medical Mycology and Parasitology, Sari, Iran
[2]Service de Microbiologie- Paris- France-, Université Paris-Descartes- Faculté de Médecine- APHP- Hôpital Européen Georges Pompidou- Unité de Parasitologie-Mycologie, Paris, France
[3]Mazandaran University of Medical Sciences- Sari- Iran-, Department of Medical Mycology and Parasitology of Medicine-, Sari, Iran
[4]Mazandaran University of Medical Sciences- Sari- Iran, Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Sari, Iran
[5]1Department of Medical Mycology and Parasitology /Invasive Fungi Research Centre IFRC- School of Medicine,
1Department of Medical Mycology and Parasitology /Invasive Fungi Research Centre IFRC- School of Medicine-, Sari, Iran

Backgrounds

ATTAF-1 and ATTAF-2 are triazole alcohol-derived analogues, containing (2,4-dichlorophenyl)-1,2,4-triazole-thiol moiety being developed for the treatment of invasive *Candida* infection. ATTAF-1 and ATTAF-2 have demonsrrated potent *in vitro* activitiy against a broad range of *Candida* species. Herein, we have evaluated the *in vivo* efficacy of ATTAF-1 and ATTAF-2 against *Candida albicans* strains in an invasive candidiasis murine model.

Objectives

We have evaluated the *in vivo* efficacy of ATTAF-1 and ATTAF-2 against *Candida albicans* strains in an invasive candidiasis murine model.

Methods

Female 4-5 weeks old CD1 (ICR) mice were used. A clinical isolate of *C. albicans*, obtained from blood culture, was used in this experiment. The day of infection, mice were challenged i.v. with 1×10^6 CFU/animal of *C. albicans* into the lateral tail vein. Groups consisting of 15 immunocompetent mice were administered ATTAF-1, ATTAF-2, and fluconazole (3.5 and 35 mg/kg/day) intraperitoneally, once daily for 5 days. The efficacy of therapy was evaluated through survival time, the fungal tissue burden and histopathological studies. Data analysis was performed by using GraphPad Prism software.

Conclusions

Mortality was significantly delayed in mice that were administered ATTAF-1 and ATTAF-2 at a dose of 35 mg/kg compared with that in the fluconazole 35 mg/kg and control mice. Fungal burden was 4.8 ± 0.36 log mean CFU/g of kidney for control mice and 2.8 ± 0.31, 2.8 ± 0.31, and 3.4 ± 0.31 for mice treated with ATTAF-1, ATTAF-2, and fluconazole (35 mg/kg), respectively.Potential therapeutic dose of ATTAF-1 and ATTAF-2 (35 mg/kg) were effective in treating invasive candidasis caused by *C.albicans* in mice. Further studies with more isolates of Fluconazole-Susceptible and -Resistant *Candida* Species representing a wider range of MICs should be carried out to assess whether there is any relationship between MIC values and ATTAF-1 and ATTAF-2 efficacy.

FEMS7-2956
Free Subjects / Other

FIRMICUTES WITH LPS-OUTER MEMBRANES: IMPLICATIONS FOR THE EVOLUTION OF CELL ENVELOPES IN BACTERIA

S. Gribaldo[1]
[1]*Pasteur, Microbiology, Paris, France*

Backgrounds

The bacterial envelope is one of the oldest and most essential cellular components. However, one of the major unanswered questions in Evolutionary Biology is why there are two substantially different cell envelope architectures, Gram-positive (monoderm) and Gram-negative (diderm), and how such dramatic transition occurred in Bacteria.
The Firmicutes represent the textbook examples of classical Gram-positive cell envelope architecture. Surprisingly, two lineages within the Firmicutes -the Negativicutes and the Halanaerobiales- display typical diderm cell envelopes with an outer membrane containing lipopolysaccharide, constituting an evolutionary conundrum.

Objectives

Our objectives were:
to clarify the evolutionary relationships among the Negativicutes and the Halanaerobiales within the Firmicutes.
to use genomic information to provide functional inference on key OM systems in these two diderm Firmicutes lineages.
to understand the evolutionary relationships of the OM of Negativicutes and Halanaerobiales with the other diderm bacterial phyla.

Methods

We carried our an exhaustive phylogenomic analysis on hundreds of complete genomes from Firmicutes and representative of major bacterial phyla. These data were confirmed and extended by experimental characterisation of the cell envelope of a diderm Firmicute.

Conclusions

Together, our results support the hypothesis that the LPS-OMs of Negativicutes and Halanaerobiales are remnants of an ancient diderm cell envelope that was present in the ancestor of the Firmicutes, and that the monoderm phenotype in this phylum is a derived character that arose multiple times independently through OM loss.
Diderm Firmicutes represent promising new experimental models to study the evolution and function of both monoderm and diderm bacterial cell envelopes.

FEMS7-3169
Free Subjects / Other

IMPROVING AMINOGLYCOSIDES ANTIBIOTICS ACTIVITY BY LIPOSOMAL FORMULATIONS: FORMULATIONS CHARACTERIZATION/ ANTIBACTERIAL STUDIES

M. Halwani[1]
[1]*King Abdullah International Medical Research Center, Nanomedicine, Riyadh, Saudi Arabia*

Backgrounds

Aminoglycoside antibiotics remain among significant antibiotics families who's still fighting microorganisms until nowadays, though, pathogenic microorganisms never settle the battle yet. Pathogenic microorganisms have used various resistance's mechanisms to avoid aminoglycoside antibiotics' kill including modifying bacterial cell membrane permeability, promoting efflux pumps, inactivating antibiotics binding sites, and producing bacterial biofilms. Nevertheless, the uncontrolled usage of these antibiotics frequently associated with adverse side effects which occurs on treated patients as general cytotoxicity or particular nephrotoxicity and ototoxicity. All above render aminoglycosides antibiotics use to be hazardous. Liposomes drug delivery systems were used for decades to improve drugs activity and reduce toxicity.

Objectives

This communication is about presenting the experience of a decade in encapsulating different aminoglycoside antibiotics inside different liposomal formulations.

Methods

Basically, Amikacin, gentamicin, and tobramycin were chosen individually in several studies to compare free forms of these antibiotics versus encapsulated forms within liposomal formulations. In addition, tobramycin was further enhanced to eradicate biofilm bacteria by co-encapsulation with bismuth-ethanedithiol in liposomal formulation. Gentamicin was also enhanced in same manner with gallium metal. Neutral and negative charged liposomes have been used recently to increase the encapsulation efficiency of gentamicin antibiotic and their antibacterial activities were assessed.

Conclusions

In conclusion, liposomal formulations as drug delivery systems apparently it can be one of the promising choices to lead pharmaceutical approach for developing conventional antibiotics such as aminoglycoside antibiotics.

FEMS7-1077
Free Subjects / Other

VALUE OF MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT FOR ROUTINE IDENTIFICATION OF VIRIDANS GROUP STREPTOCOCCI

G. Hasçelik[1], O. Altay[2], N. Karacan[3], M. Özdal Salar[3], H.K. Müstak[3]
[1]Hacettepe University Faculty of Medicine, Ankara, Turkey
[2]Hacettepe University Faculty of Medicine, Department of Medical Microbiology, Ankara, Turkey
[3]Ankara University Faculty of Veterinary Medicine, Department of Microbiology, Ankara, Turkey

Backgrounds

Phenotypic tests do not always unequivocally identify some species viridans group streptococci (VGS). In the last couple of decades, molecular methods have enabled more accurate genotypic identification of bacteria. In recent years, the matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) technique has emerged as an alternative for clinical microbiology laboratories for the identification of a wide range of bacteria and fungi.

Objectives

This study assesses the ability of MALDI Biotyper to identify VGS isolates using 16S rDNA sequence analysis.

Methods

All the 94 clinical isolates of VGS were identified by conventional microbiological methods and then by MALDI Biotyper (Bruker Daltonics, Germany). The spectra were analysed by using the Flex Control 3.0 software and MALDI Biotyper 3.1 database library. 16S rRNA gene sequencing was used as a reference method for species identification.

Results: MALDI Biotyper accurately identified 78.7%(n:74) of the isolates to species level. Only one of the 94 isolates was misidentified as *S. pneumoniae* by the MALDI Biotyper system .
Maldi Biotyper (n=30; 21 S. oralis and 9 S. mitis) were able to distinguish S. mitis/oralis. 16S rDNA analysis failed to identify three strains and named these as S. mitis/S.oralis.

Conclusions

MALDI-TOF appeared to be a rapid and reliable alternative for identification of VGS strains to group level, but was not able to discriminate closely related species of certain groups.

FEMS7-1820
Free Subjects / Other

IS THERE ANY CORRELATION BETWEEN BIOFILM PRODUCTION AND ANTIBIOTIC SUSCEPTIBILITY TESTS OF BURKHOLDERIA SPECIES ISOLATED FROM CYSTIC FIBROSIS AND NON-CYSTIC FIBROSIS PATIENTS?

O. Altay[1], G. Hascelik[1], B. Sener[1]
[1]Hacettepe University- Faculty of Medicine, Department of Microbiology, Ankara, Turkey

Backgrounds

Biofilm formation is an important virulence factor of various types of bacteria. Biofilm matrix provides a protective shield against the host immune system, besides it forms a physical barrier to the penetration of antibiotics.

Objectives

The aim of this study was to compare the biofilm formation of *Burkholderia* spp. strains isolated from CF and non-CF patients and to evaluate the correlation between *biofilm production* and antibiotic resistance.

Methods

A total of 36 *Burkholderia* spp. (15 CF, 13 non-CF and 8 control strains [LMG 1222, LMG 21824, LMG 16654, LMG 18829, LMG 18157, LMG 16775, LMG 13010 and LMG 1883]) isolates were analyzed. All the *Burkholderia* spp. were identified by conventional methods and MALDI-TOF-MS method (Bruker-Biotyper, USA). Biofilm formation was determined by using crystal violet microtiter plate assay. Antibiotic susceptibilities of ceftazidim, meropenem and trimethoprim/sulfamethoxazole (TMP-SXT) were determined by disc diffusion method according to CLSI guidelines.

Results: Among the 28 *Burkholderia* spp. isolates, biofilm production was determined in 78.5% (22/28) of the test isolates. No significant difference was detected among the biofilm positivity rates between CF (80%, 12/15) and non-CF (76.9%, 10/13) *Burkholderia* isolates. Rates of resistance to ceftazidime were 36.7% and 61.5%; to meropenem were 53.3% and 15.9%; to TMP-SXT were 0% and 15.9%, respectively, in CF and non-CF isolates.

Conclusions

No significant correlation between *biofilm production* and antibiotic resistance were detected. *Burkholderia* spp. exhibits significant susceptibility to TMP-SXT. Higher meropenem resistance in CF patients might be attributed to the expression of the specific efflux pump in the CF context.

FEMS7-2978
Free Subjects / Other

SEROTYPE DISTRUBITION IN TRANSPOSON CARRYING MULTI-DRUG RESISTANT STREPTOCOCCUS PNEUMONIAE AMONG INVASIVE AND NON-INVASIVE ISOLATES

F.N. Akdogan Kittana[1], I.B. Kaya[2], G. Hascelik[1], S. Saricam[2], N. Gurler[3], K.S. Diker[2]
[1]University of Hacettepe Faculty of Medicine, Department of Medical Microbiology, Ankara, Turkey
[2]University of Ankara Faculty of Veterinary Medicine, Department of Microbiology, Ankara, Turkey
[3]University of Istanbul Faculty of Medicine, Department of Medical Microbiology, Istanbul, Turkey

Backgrounds

Streptococcus pneumoniae infections are challenging since pneumococci have more than 90 serotypes and its emergence of resistant strains has increased. The widespread dissemination of antibiotic resistance among pneumococci is associated with horizontal gene transfer (HGT) with mobile genetic elements like transposons. Transposons cause higher risk of treatment failure in pneumococcal infections.

Objectives

The aim of this study was to detect serotype distribution and transposons of multi-drug resistant invasive and non-invasive pneumococcal isolates.

Methods

Sixty-four invasive and 43 non-invasive isolates were included. For serogrouping and serotyping of the isolates, latex particule agglutination and Quellung reaction were performed, respectively. The resistance genes (*erm*A, *erm*B, *mef*E, *mef*I, *tet*M, *tet*O, *aph*A-III and *cat*pC194) and Tn916 transposons specific genes (*int*, *xis*, *tnp*A and *tnp*R) were investigated by *in*-house PCR with spesific primers. The results were compared with already known transposons.

Conclusions

Serotype 19F and 19A were first and second commonly observed in invasive (40.6% and 15.62%) and non-invasive (39.5% and 6.98%) isolates, respectively. Majority of the pneumococci (88.2%) carries Tn*916* family's transposons. Most commonly detected transposon was Tn*2010* (37.2%). Transposon Tn6002 (28.12% and 13.95%) and Tn3872 (15.62% and 20.9%) were detected in invasive and non-invasive isolates, respectively. Serotype distribution and transposon types have no statistically significant difference between invasive and non-invasive isolates. The 13 and 23 valent pneumococcal vaccine covered the studied isolates. Since serotype distribution and transposons of *S.pneumoniae* isolates may change in time naturally, close monitoring is essential.

FEMS7-0738
Free Subjects / Other

FREQUENCY AND ANTIMICROBIAL SENSITIVITY OF ESCHERICHIA COLI 0157:H7 IN HEALTHY FARM/ABATTOIR/POULTRY WORKERS OF PESHAWAR, PAKISTAN

A. Jan[1], J. Ahmed[1], S. Mumtaz[2]
[1]Institute of Basic Medical Sciences- Khyber Medical University, Microbiology, Peshawar, Pakistan
[2]Khyber Medical College, Pathology, Peshawar, Pakistan

Backgrounds

Escherichia coli O157:H7 causes severe food-borne diseases transferred through milk, beef, and mutton. It mainly causes diseases in people taking these foods and milk coming from animals and poultry.

Objectives

This study was conducted to determine whether this organism is transferred through healthy humans associated with animals and poultry to the community. Therefore zoonosis through *Escherichia coli* O157:H7 was determined in healthy farm, abattoir and poultry workers in Peshawar.

Methods

The data were collected through standardized questionnaire in which questions were based on the hygiene practice, work duration, exposure to unprescribed antibiotics and disease history. A total of 200 samples were collected were collected including swabs from hands and mouth and from surfaces frequently used to work and stool sample, out of which 50(25%) samples were taken each from farm, poultry and abattoir workers while the rest of 50 (25%) samples were taken from the regular visitors of the sites. Stool and swab samples were collected from healthy workers.

Conclusions

Prevalence of *E. coli* O157:H7 was calculated as 23(11.5%) isolates recovered from farm workers, 21(10.5%) from abattoir workers, 20(10%) from poultry workers and 19 (9.5%) from visitors of these areas. The overall prevalence calculated was 41.5 per hundred persons. The organism was resistant to Vancomycin and sensitive to Ciprofloxacin. Four primers were used but amplification was observed on primers for Shiga toxin 3 and 4.

The organism is highly prevalent in farm and abattoir workers and they are considered to be active carriers of E. coli O157:H7.

FEMS7-0803
Free Subjects / Other

AN ESSENTIAL ROLE FOR TAGLN2 IN PHAGOCYTOSIS OF LIPOPOLYSACCHARIDE-ACTIVATED MACROPHAGES

C.D. Jun[1]
[1]Gwangju Institute of Science and Technology, School of Life Sciences, Gwangju, Republic of Korea

Backgrounds

Bacterial infection is a common cause of sepsis and sepsis-associated organ failure, which cause high morbidity and mortality. Macrophages are the first line of host defense against pathogens and phagocytosis is one of the most important initial immune responses. The polymerization actin cytoskeleton mediates protrusions or ruffling of the macrophage membrane that can internalize target pathogens. In this process, a large number of actin-regulatory proteins are participating in actin remodeling. However, the molecular machineries that account for the enhanced ability of phagocytosis in activated macrophages as compared to resting macrophages are relatively unknown.

Objectives

We investigated the role of TAGLN2, a 22-kDa actin-binding protein, in Toll-like receptor (TLR)-stimulated phagocytosis.

Methods

TAGLN2 expression level was examined using reverse transcriptase quantitative polymerase chain reaction, and Western blotting. To examined whether function of TAGLN2 is related with the receptor-mediated phagocytosis, we utilized macrophages obtained from TAGLN2-deficient (*TAGLN2*$^{-/-}$) mice. For ex vivo and in vivo phagocytosis, we used E.coli and Salmonella and phagocytic activity was determined by flow cytometry and confocal real-time live imaging using confocal microscopy.

Conclusions

Here, we demonstrate TAGLN2 was greatly induced in macrophages in response to lipopolysaccharide (LPS), a ligand for TLR4, partly via the NF-κB pathway. TAGLN2-deficient macrophages (*TAGLN2*$^{-/-}$) showed defective phagocytic functions of IgM- and IgG-coated sheep red blood cells as well as bacteria. Cell signaling pathways involved in actin rearrangement—PI3 kinase/AKT and Ras-ERK—were also down-regulated in LPS-stimulated TAGLN2-deficient macrophages. Moreover, *TAGLN2*$^{-/-}$ mice showed higher mortality after bacterial infection than wild-type littermates. Thus, current results provide a novel function of TAGLN2 as a molecular armament required for host defense.

FEMS7-1850
Free Subjects / Other

INVESTIGATING THE EFFICACY OF DISINFECTANTS BEING USED IN A HOSPITAL AGAINST SOME NOSOCOMIAL INFECTION CAUSING BACTERIA

Ç. Yavaş[1], F. Kaynak Onurdağ[2]
[1]Trakya University Institute of Health Sciences, Department of Basic Pharmaceutical Sciences, Edirne, Turkey
[2]Trakya University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Edirne, Turkey

Backgrounds

Hospital infections are severe infections with high mortality and morbidity. However, they are preventable infections when appropriate precautions are taken. Disinfectant activity tests are applied with standard bacterial strains and bacterial strains isolated from hospital environments may have different sensitivities than their standard counterparts.

Objectives

In our study, it was aimed to determine the efficacy of the disinfectants used in Trakya University Hospital to the bacteria isolated from the same hospital.

Methods

Twenty-five *Acinetobacter baumannii* (resistant to ciprofloxacin, gentamicin, meropenem, imipenem and ceftazidime), 14 *Klebsiella pneumoniae* (ESBL), 15 *Staphylococcus aureus* (MRSA) and 14 *Enterococcus faecalis* (VRE) isolates were included in the study. Susceptibilities were tested through the guidelines of CLSI M100-S25.

OPASTER®, SEKUSEPT®, MOONCID® PULVEREX, INCIDIN® Foam, ANIOS® Aniospray Quick, ANIOS® Aniosrub, OXY®, OXY®-WC-Bath, EXPÜR® and PENTAX® were tested with the applied ways in the hospital in clean and dirty conditions through TS EN 1040/1999 guidelines by qualitative suspension test.

Conclusions

As a result; all bacteria were observed to be susceptible to Incidin®Foam, Anios®Aniospray, Anios®Aniosrub, Oxy® ve Oxy®WC-Bath in all conditions and to be susceptible to Opaster® in hospital conditions. However, resistant isolates were observed to Ekusept®, Expür® and Pentax®.

Three disinfectants do not have efficacy to the isolates and this may lead to a spread in the hospital infections which also cannot be treated. As a result, we recommend that these disinfectants should not be used in our hospital and disinfectant efficacy tests should be applied to the drug resistant isolates in the hospitals regularly.

FEMS7-1375
Free Subjects / Other

IN VITRO SUSCEPTIBILITY PATTERNS OF CLINICALLY IMPORTANT TRICHOPHYTON AND EPIDERMOPHYTON SPECIES AGAINST NINE ANTIFUNGAL DRUGS

S. Khodavaisy[1], H. Badali[2], H. Fakhim[2]
[1]Tehran University of Medical Sciences, Department of Medical Mycology and Parasitology, Tehran, Iran
[2]Mazandaran University of Medical Sciences,
Department of Medical Mycology and Parasitology/Invasive Fungi Research Center IFRC- School of Medicine, sari, Iran

Backgrounds

Despite the common, worldwide, occurrence of dermatophytes, little information is available regarding susceptibility profiles against currently available and novel antifungal agents.

Objectives

In the present investigation, we evaluated the in vitro antifungal susceptibilities of nine antifungal agents, viz. amphotericin B, fluconazole, itraconazole, voriconazole, isavuconazole, posaconazole, terbinafine, anidulafungin and caspofungin against sixty-eight clinical Trichophyton species and Epidermophyton floccosum.

Methods

A collection of sixty-eight clinical Trichophyton species and Epidermophyton floccosum were previously identified and verified to the species level by sequencing the internal transcribed spacer (ITS) regions of rDNA. MICs of amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, terbinafine and MECs of caspofungin and anidulafungin were performed based on CLSI M38-A2.

Conclusions

The resulting MIC90s of all strains were, in increasing order, as follows: terbinafine (0.063 mg l1); posaconazole (1 mg l1); isavuconazole and anidulafungin (2 mg l1); itraconazole, voriconazole, amphotericin B, and caspofungin (4 mg l1) and fluconazole (>64 mg l1). These results confirm that terbinafine is an excellent agent for treatment of dermatophytosis due to T. rubrum, T. mentagrophytes, T. verrucosum, T. schoenleinii and E. floccosum. In addition, the new azoles POS and ISA are potentially useful antifungals to treat dermatophytosis. However, the clinical effectiveness of these novel antifungals remains to be determined.

FEMS7-1383
Free Subjects / Other

MOLECULAR CHARACTERIZATION OF HIGHLY SUSCEPTIBLE CANDIDA AFRICANA FROM VULVOVAGINAL CANDIDIASIS

S. Khodavaisy[1], *H. Badali*[2], *S. Mohsenii*[3], *S. Olia*[4]
[1]*Tehran University of Medical Sciences, Department of Medical Mycology and Parasitology, Tehran, Iran*
[2]*Mazandaran University of Medical Sciences, Department of Medical Parasitology and Mycology, Sari, Iran*
[3]*Sari Branch-, Department of Microbiology, Sari, Iran*
[4]*Gazvin University of Medical Sciences, Department of Medical Mycology and Parasitology, Gazvin, Iran*

Backgrounds

Phylogenetic studies highlight Candida africana as an atypical variant within Candida albicans species complex which is dominantly recovered from vaginal specimens.

Objectives

This study aimed to characterize *C. africana* isolates from patients with vulvovaginal candidiasis (VVC) by molecular methods and in vitro susceptibilities.

Methods

One hundred and fifty-six (48.44 %) Candida strains were collected from 322 patients diagnosed with VVC. Of these, 114 (73.07 %) were germ tube positive and presented green color on the chromogenic medium, thus classified as *C. albicans* species complex.

Conclusions

One hundred and nine (95.61 %) out of 114 isolates were identified as *C. albicans*, while five (4.38 %) isolates were identical with C. africana based on hwp1 PCR. C. africana appeared to be highly susceptible to the tested antifungals. In conclusion, among the *C. albicans* species complex, *C. albicans* predominantly and *C. africana* rarely occur in vaginal mucosa. Due to limited information on molecular epidemiology of this novel yeast, more studies using molecular methods are needed to elucidate the interand intraspecific genomic variations of *C. africana* isolates.

FEMS7-0324
Free Subjects / Other

CORALMYCINS A AND B, NEW POTENT ANTI-GRAM NEGATIVE COMPOUNDS FROM THE MYXOBACTERIA CORALLOCOCCUS CORALLOIDES M23

W.G. Kim[1], Y.J. Kim[1], H.J. Kim[1], K. Cho[2], H. Koshino[3]
[1]Korea Research Institute of Bioscience & Biotechnology, Superbacteria Research Center, Daejeon, Republic of Korea
[2]Hoseo University, Department of Biotechnology, Asan, Republic of Korea
[3]RIKEN, Center for Sustainable Resource Science, Wako, Japan

Backgrounds

Recently, infections caused by MDR Gram-negative bacteria have become a growing problem. There is an urgent need for new agents to treat infections caused by Gram-negative bacteria resistant to currently available agents.

Objectives

Myxobacteria are a group of Gram-negative bacteria that produce a diverse range of bioactive secondary metabolites. Myxobacteria have received attention as a source of novel anti-infective natural products. Our objective is to search for anti-Gram-negative metabolites from Korean myxobacteria.

Methods

We report the producing strain, fermentation, isolation, structural determination, and antibacterial activities of potent anti-Gram negative compounds.

Conclusions

Two new potent anti-Gram negative compounds, coralmycins A (**1**) and B (**2**), were isolated from cultures of the myxobacteria *Corallococcus coralloides* M23, together with another derivative (**3**) that was identified as the very recently reported cystobactamid 919-2. Their structures including the relative stereochemistry were elucidated by interpretation of spectroscopic, optical rotation, and CD data. The relative stereochemistry of **3** was revised to *"S*R*"* by NMR analysis. The antibacterial activity of **1** was most potent against Gram-negative pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Klebsiella pneumoniae*, with MICs of 0.1–4 μg/mL; these MICs were 4–10 and 40–100 times stronger than the antibacterial activities of **3** and **2**, respectively. Thus, these data indicated that the β-methoxyasparagine unit and the hydroxy group of the benzoic acid unit were critical for antibacterial activity. Coralmycin A has great potential for treatment of multidrug-resistant bacteria, including Gram-negative bacteria.

FEMS7-0763
Free Subjects / Other

SYNERGISTIC EFFICACY OF SODIUM NEW HOUTTUYFONATE IN COMBINATION WITH BERBERINE AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

X. Li[1], C. Li[1], X. Lu[1], J. Pang[1], X. You[1], X. Yang[1]
[1]Chinese Academy of Medical Sciences & Peking Union Medical College, Institute of Medicinal Biotechnology, Beijing, China

Backgrounds

Staphylococcus aureus is a normal colonizing species in the human anterior nares and causes a wide variety of diseases. It is notorious for its ability to become resistant to antibiotics, thus limiting treatment options. Plants have provided us with a good source of antimicrobial agents and are candidates as new antibiotic substances.

Objectives

We evaluated two natural compounds, sodium new houttuyfonate (SNH) and berberine, for their in vitro efficacy against Methicillin-Resistant *Staphylococcus aureus* (MRSA).

Methods

A total of 162 *S. aureus* clinical isolates were collected from hospitals in Beijing, China, from 2009-2013. MRSA isolates were identified and screened by VITEK 2-COMPACT system, MIC of Oxacillin, and a multiplex polymerase chain reaction (PCR) targeting the 16S ribosomal RNA (rRNA) and mecA. MIC and checkerboard assay were performed to test antibacterial activity of SNH and berberine individually or in combination, respectively. Time killing analysis was performed to characterize the kill kinetics of SNH and berberine both alone and in combination against selected clinical isolates and well characterized control strains.

Conclusions

SNH inhibited all test strains with minimum inhibitory concentrations (MICs) ranging from 16 to 64 µg/mL in susceptibility tests, while 32-512 µg/mL for berberine alone. SNH and berebreine inhibited bacterial growth in a concentration-dependent manner in time-kill analysis, and showed significant synergistic effects against *S. aureus* Mu50, ATCC 43300, ATCC33591 and clinical isolated 8-50. Our findings suggest that the combination of two natural compounds, SNH and berberine, is a promising candidate for anti-MRSA drug development.

FEMS7-2590
Free Subjects / Other

‘CREDIBLE’ – A CRE-MEDIATED DOUBLE REPORTER SYSTEM TO STUDY PENETRATION OF PHYSIOLOGICAL BARRIERS BY CELL-PENETRATING EFFECTOR PROTEINS

M.L. Lubos[1], T. Danyukova[2], B. Skryabin[3], C. Geyer[4], M.A. Schmidt[1], C. Rüter[1]
[1]Institute of Infectiology, Center for Molecular Biology of Inflammation, Münster, Germany
[2]Center for Obstetrics and Pediatrics, Department of Pediatrics, Hamburg, Germany
[3]TRAnsgenic animal and genetic engineering Models TRAM, Medical Faculty, Münster, Germany
[4]IZKF Core Unit "Preclinical Imaging eXperts" PIX, Institute of Clinical Radiology, Münster, Germany

Backgrounds

Previous studies identified the effector protein YopM of *Yersinia enterocolitica* as a novel bacterial cell-penetrating effector (CPE). The two N-terminal α-Helices (2αH) of YopM mediate translocation across the host cell plasma membrane independently of *Yersinia*’s type three secretion system (T3SS) and intracellular delivery of molecular cargos like GFP.

Objectives

In order to further study and characterise the mechanisms of cell penetration by CPEs and further cell-penetrating peptides (CPPs) *in vivo*, we have generated a Cre-mediated double reporter (‘CREDIBLE’) system. Transgenic mice harbouring the ‘CREDIBLE’ construct, express two reporter genes, namely near-infrared fluorescent protein (iRFP) and luciferase upon Cre/loxP-recombination.

Methods

The ‘CREDIBLE’ system is functional and both reporters are expressed upon recombination *in vitro*. Furthermore, crossing transgenic mice with mice expressing Cre-recombinase leads to recombination events, indicating the functionality of the system *in vivo*.

To analyse the distribution of CPP/CPEs *in vivo*, we have constructed different CPP-Cre fusion proteins, including 2aH-Cre. The recombinant proteins will be administered to the transgenic mice via different routes and their distribution can be analysed in real time by non-invasive live optical imaging.

Conclusions

Additionally, the ‘CREDIBLE’ system can be applied in a variety of studies using drug delivery systems like exosomes or targeted drug delivery to characterise their efficacy and distribution *in vivo*. Furthermore, the ‘CREDIBLE’ system can be used to monitor bacterial or viral infections *in vivo* and, in particular, to gain a more detailed insight into the role and function of various virulence factors during infection (e.g. secreted bacterial effector proteins, outer membrane vesicles).

FEMS7-0467
Free Subjects / Other

ONE DAY-OLD CHICKS AS A SOURCE OF ANTIMICROBIAL RESISTANT BACTERIA FOR COMMERCIAL LAYING HENS FARMS

M.A. Moreno[1], M. Ugarte[2], C. Bárcena[2], M. García[2], F. Esperón[3], J.L. Tadeo[4], L. Domínguez[2]
[1]Universidad Complutense de Madrid, Departamento de Sanidad Animal, Madrid, Spain
[2]Universidad Complutense de Madrid, Centro de Vigilancia Sanitaria Veterinaria, Madrid, Spain
[3]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Centro de Investigación en Sanidad Animal, Valdeolmos, Spain
[4]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Departamento de Medio Ambiente, Madrid, Spain

Backgrounds

Some antimicrobial resistant (AR) *Salmonella* spp. are detected on the national Spanish monitoring programmes in laying hens (LH) in spite of the few antimicrobials authorized for LH in Spain. Testing of AR on *Escherichia coli* from LH is not mandatory at European level but it is clear that AR on *E. coli* should be not lower than that on *Salmonella* spp., having besides a higher prevalence. Accordingly, *E. coli* is a good model for testing sources of AR bacteria in commercial LH farms.

Objectives

To test if AR *E. coli* isolates are introduced into a commercial LH farm by one day-old chicks and to follow its dynamics until entering table egg production.

Methods

Three batches of one day-old chicks from different hatcheries purchased by a commercial LH farm were sampled three times: when arriving to the farm (samples from 15 transport boxes) , at two weeks of age (samples from 10 manure belts) and some days before change to the laying house (18 weeks). Samples where pooled, diluted with peptone water and spread onto MacConkey agar plates (incubation at 37 ºC for 20-24 h). Ten presumptive *E. coli* colonies per sampling where picked, identified by PCR and AR determined using EUVSEC plates (Sensititre ®) containing 14 antimicrobials.

Conclusions

There are clear differences on AR *E. coli* (multiresistance patterns and resistance frequencies) between one day-old chicks batches from different suppliers; nevertheless, these indicators of AR tend to down at the end of the growing phase.

This work was funded by RTA2014-00012-C03-03.

FEMS7-2466
Free Subjects / Other

3-D CELL CULTURE METHODS TO ACHIEVE ANTI-CANCER DRUG

F.S. Mousavi[1], R. Heidarifar[2], H. Jamshidi[3], A. Daemi[1]
[1]Urmia Branch -Islamic Azad Univercity, Biotechnology, Urmia, Iran
[2]shahid beheshti university, medical school, teheran, Iran
[3]kermanshah university, medical school, kermanshah, Iran

Backgrounds

Many animal cells can be propagated outside the animal body. The isolated cells from the tissues and organs of animals may be at the right temperature in a medium containing nutrients and growth factors to grow into plastic containers. Cultured organs, tissues and cells of organisms in vitro cell culture is called two different applications in different branches of biological sciences

Objectives

Recent studies have demonstrated the potential of utilizing 3D cell culture models in drug discovery programs; however, it is evident that further research is required for the development of more complex models that incorporate the majority of the cellular and physical properties of a tumor.

Methods

The methods for culturing cells in 3D utilizing lrECM as a subtrate involves seeding a single cell suspension either on top of matrices (3D ‘on top’ assay) or mixed into lrECM (3D ‘embedded’ assay), which promotes the formation of cells into 3D structures in a time-dependent manner . LrECM is not the only biologically relevant matrix available for 3D cell culture. Collagen I has also been utilized as a substrate for culturing tumor cells in 3D systems.

Conclusions

A clearer understanding of the complex mechanisms influencing the mode of action and efficacy of cancer therapeutics is essential to move closer towards the goal of eradicating cancer cells in the patient. Research into new 3D tumor models that more closely represent the tumor microenvironment in systems that are scalable to meet the requirements of screening practices is underway, with notable progress published recently

FEMS7-0862
Free Subjects / Other

INTERFERENCES IN CLINICAL DIAGNOSIS CAUSED BY THE PRESENCE OF INFECTIOUS BACTERIOPHAGES IN THE HUMAN BODY

B.J. Maryury[1], F. Navarro[2], M. Muniesa[1]
[1]Universitat de Barcelona, Genetic- Microbiology and Statistics, BARCELONA, Spain
[2]Hospital de la Santa Creu i Sant Pau- Institut d'Investigació Biomèdica Sant Pau, Service of Microbiology, Barcelona, Spain

Backgrounds

Bacteriophages have re-emerged as powerful regulators of the bacterial populations in natural ecosystems. As in natural environments, phages invade the human body and they are the most numerous group of virus in human viromes of healthy individuals. This is revealed in recent metagenomic studies, despite the presence of phages in human bodies was described decades ago. The influence of the presence of phages in humans has yet to be evaluated, but it could be envisaged a clear influence in the regulation of bacterial populations, that might have later an impact in human health.

Objectives

To explore the presence of phages in clinical samples and to evaluate how these phages could interfere in clinical diagnosis.

Methods

Clinical samples (blood, ascitic, urine, cerebrospinal fluid and serum) were evaluated for the presence of infectious bacteriophages able to propagate and lyse *E. coli* and *Pseudomonas* host strains. The presence of phage particles was confirmed by electron microscopy.

Conclusions

Infectious tailed phages were detected in >46% ascitic and urine samples. The presence of phages was shown to hinder bacterial isolation in some of these samples, either by preventing the confluent bacterial growth required for an antibiogram assay, by reducing the bacterial growth in liquid enrichment or by killing the bacteria present in a sample, confounding the diagnostic. The presence of phages in human samples, most of the times not considered, can influence and bias the results of microbiological and molecular results, suggesting that more attention should be paid to their interference.

FEMS7-2258
Free Subjects / Other

THE ROLE OF MAGNETOSOMES ON THE TOLERANCE TO HEAVY METALS IN MAGNETOTACTIC BACTERIA

D. Muñoz-Rodríguez[1], L. Marcano[2], R. Martín-Rodríguez[3], A. García Prieto[4,5], J. Alonso[2,5], M.L. Fdez-Gubieda[2,5], A. Muela[1,5]
[1]Universidad del País Vasco UPV/EHU, Inmunología- Microbiología y Parasitología, Leioa, Spain
[2]Universidad del País Vasco UPV/EHU, Electricidad y Electrónica, Leioa, Spain
[3]Universidad de Cantabria, QUIPRE, Santander, Spain
[4]Universidad del País Vasco UPV/EHU, Física Aplicada I, Leioa, Spain
[5]BCMaterials, Building No.500- Parque Tecnológico de Zamudio, Derio, Spain

Backgrounds

Magnetotactic bacteria are a group of aquatic prokaryotes capable of navigating along the Earth´s magnetic field lines thanks to the production of internal magnetic nanoparticles called magnetosomes. These nanoparticles, whose synthesis is genetically controlled, are the subject of intensive research because of their well-defined magnetic properties, becoming really useful in biotechnological applications (1). Currently, the generally accepted role of magnetosomes is based on magnetoaerotaxis hypothesis: these magnetosomes favour the alignment of the bacteria with the geomagnetic field lines, allowing them move in a chemical redox gradient in a more efficient way within the water column (2). However, it is still not clear if the magnetic navigation is the only purpose of producing such organelles.

Objectives

In the present work, we explore a new possible role of magnetosomes as `protective shields´ against metal stress induced by the presence of heavy metals.

Methods

With this aim we have determined the resistance profiles to several transition metals (Co, Mn, Ni, Zn and Cu) in *Magnetospirillum gryphiswaldense* MSR-1, either when bacteria present magnetosomes or not. We have observed that the tolerance to the different metals is significantly higher (up to six-fold higher in Mn) when the bacteria present magnetosomes. Moreover, we have proved by magnetic and structural characterization techniques the incorporation of some of these elements into the magnetosome structure.

Conclusions

The incorporation of metals other than Fe into the magnetosome structure opens up the possibility of using magnetotactic bacteria as a tool in bioremediation applications such as biomagnetic recovery of metals from raw sewage (3).

(1) E Alphandéry. Front. Bioeng. Biotechnol, 2014.
(2) D. A Bazylinski & R. B Frankel. Nature Rev. Microbiol, 2004.
(3) M Tanaka et al., Appl. Environ. Microbiol, 2016.

FEMS7-2520
Free Subjects / Other

THE EFFICIENCY OF MAGNETOSOMES IN MAGNETIC HYPERTHERMIA FOR CANCER TREATMENT

A. Muela[1,2], D. Muñoz[1], R. Martín-Rodríguez[3], I. Orue[4], E. Garaio[3], A. Abad Díaz de Cerio[1], J. Alonso[2], J.Á. García[2,5], M.L. Fdez-Gubieda[2,3]
[1]Universidad del País Vasco, Inmunología- Microbiología y Parasitología, Leioa, Spain
[2]BCMaterials, No.500 Building- Parque Tecnológico de Zamudio, Derio, Spain
[3]Universidad del País Vasco, Electricidad y Electrónica, Leioa, Spain
[4]Universidad del País Vasco, SGIker Medidas Magnéticas, Leioa, Spain
[5]Universidad del País Vasco, Física Aplicada II, Leioa, Spain

Backgrounds

Magnetotactic bacteria are ubiquitous aquatic prokaryotes able to synthesize intracellular membrane-bound magnetite or greigite nanoparticles called magnetosomes (1). Currently, the magnetosomes provoke great attention thanks to several outstanding physical and magnetic properties (uniform morphology, thermally stable magnetic moment, high biocompatibility), that make possible their use in biomedical applications, such as magnetic resonance imaging or magnetic hyperthermia for cancer treatment. Based on the higher sensitivity of cancerous cells to temperature increase compared to healthy tissue (2), the hyperthermia treatment makes use of the heat-releasing power of magnetic nanoparticles when exposed to an alternating magnetic field (AMF) to produce cell death. Indeed, magnetosomes hold high potential for hyperthermia applications, since they have shown very promising heating capacity (3).

Objectives

In the present work, the efficiency of magnetosomes as heat generators and their impact on cell viability has been checked on murine macrophage cells in *in vitro* tests.

Methods

Our results clearly indicate that the hyperthermia treatment causes both cell death and inhibition of cell proliferation. Specifically, only 36% of the treated macrophages remained alive 2 h after alternating magnetic field exposure, and 24 h later the percentage fell to 22%.

Conclusions

This reduction confirms the suitability of the magnetosomes as efficient heat nanogenerators in hyperthermia treatments.

(1) E Alphandéry *et al*. J. Phys. Chem, 2011.

(2) D Ortega Ponce *et al.* Nanoscience, 2012.

(3) A Muela *et al*. J. Phys. Chem. C, 2016.

FEMS7-3149
Free Subjects / Other

GFP-BINDING APTAMER - A TOOL FOR RECOMBINANT PROTEINS PURIFICATION

K. Muszynska[1], E. Kowalska[1], F. Bartnicki[1], M. Bodaszewska-Lubas[1], W. Strzalka[1]
[1]Jagiellonian University, Plant Biotechnology, Krakow, Poland

Backgrounds

Green Fluorescent Protein (GFP) is often used as a tag of cellular proteins. It is very useful in a broad range of studies including detection and enumeration of bacteria, monitoring of microorganisms survival, gene expression and protein localisation. Despite the fact that GFP is a very powerful tool for prokaryote and eukaryote cells studies, the purification of GFP-tagged proteins from such material is limited to anti-GFP antibodies or other conventional chromatography methods.

Objectives

Due to popularity of GFP in cell biology research, the aim of the study was to develop a DNA aptamer, which could be used for the purification of GFP-tagged proteins from microbial, plant and animal cells.

Methods

The selection of the DNA aptamer was performed by the SELEX method (Systematic Evolution of Ligands by Exponential Enrichment). GFP used in selection process was overexpressed and purified from *E.coli* BL21 strain. After the sixth round of selection the pool of ssDNA molecules was cloned and sequenced. The specificity of single aptamers was analyzed with the help of quantitative real-time PCR. Among the tested sequences, the aptamer characterized by the best binding to GFP was identified. The specificity of this aptamer was verified using the pull-down assay.

Conclusions

The results of study suggest that the developed aptamer might serve as a new tool for the purification of GFP-tagged proteins. Further research on the selected aptamer may expand variations of implements for protein purification.

FEMS7-2336
Free Subjects / Other

PHARMACOLOGICAL ACTIVITY AND MECHANISM OF ACTION FOR SOME VEGETAL COMMERCIAL OILS ON ANTIBIOTIC RESISTANT AND BIOFILM FORMING MICROORGANISMS

H. Najee[1], C. Kamerzan[2], V. Lazar[3]
[1]Bucharest university-Faculty of Biology, Botany-Microbiology, Bucharest, Romania
[2]Research Institute of the University of Bucharest-ICUB, Microbiology, Bucharest, Romania
[3]Research Institute of the University of Bucharest-ICUB, Botany-Microbiology, Bucharest, Romania

Backgrounds

Discovering new antibacterial strategies represents a real scientific challenge, due to the antimicrobial resistance growing problem (among the eight top health global problems till 2020). Our aim was to assess the anti-pathogenic activity of the extracts from *Nigella sativa, Thymus vulgaris* and *Syzygium aromaticum* mixture (M-ST), *Origanum vulgare* and *Olea europaea* commercial oils, and to propose a mechanism of action for the tested extracts

Objectives

To investigate the biological activity of some medicinal plants extracts and essential oils on antibiotic resistant microbial strains and biofilm forming microorganisms

Methods

The clinical isolates were identified (by Vitek method) and characterized for their antibiotic resistance profile (by disc diffusion method). A set of 43 strains, belonging to four representative microbial species were studied*: Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The antimicrobial activity of the commercial extracts was assessed by using the binary serial dilution method, the concentrations ranges being oil specific. Two other techniques were used, i.e. the crystal violet staining for the anti-biofilm activity and flow cytometry, for identifying the mechanism of action for the tested extracts (cell coatings permeation, or efflux pumps inhibition).

Conclusions

Our results proved that the tested oils are valuable alternatives for the development of novel anti-pathogenic strategies. Future tests will be made for comparative analysis of the effect exhibited by commercial *vs* freshly extracted fatty and essential oils.

FEMS7-3188
Free Subjects / Other

ANTIMICROBIAL PROPERTIES OF POLYPHENOL EXTRACTS FROM TWO AUTOCHTHONOUS BEAN LANDRACES OF THE CAMPANIA REGION, SOUTHERN ITALY, BEFORE AND AFTER COOKING

F. Nazzaro[1], M.N. Ombra[1], A. d'Acierno[1], R. Coppola[2], M. Zaccardelli[3], C. Pane[3], P. Spigno[4], R. Riccardi[4], F. Fratianni[1]
[1]Istituto di Scienze dell'Alimentazione - CNR, DiSBA-CNR, Avellino, Italy
[2]DiAAA-University of Molise, DiAAA-UNIMOL, Campobasso, Italy
[3]CREA, CREA, Pontecagnano SA, Italy
[4]Cooperativa ARCA 2010, Cooperativa ARCA 2010, Acerra NA, Italy

Backgrounds

Common beans (*Phaseolus vulgaris*) represent a worthy supply of essential nutrients and phenolic compounds with resourceful health benefits. However, the biochemical and structural changes resulting from cooking processes, can, in a certain sense, alter or diminish some of such advantages.

Objectives

Polyphenol-rich extracts of two indigenous landraces of *Phaseolus vulgaris*, “Nero di Frigento” and “Nero di Acerra”, cultivated in the Campania region, Southern Italy, were analyzed, before and after cooking of beans, to determine their potential antimicrobial activity.

Methods

Antimicrobial activity was evaluated through the inhibition halo test using two pathogen strains, S*taphylococcus aureus* and *Pseudomonas aeruginosa.*

Conclusions

Polyphenolic extracts of the uncooked “Nero di Frigento” beans showed antimicrobial activity against *Ps. aeruginosa*, and a halo of 18.3 mm of diameter was visualized using 5 µg of the extract. The activity was even higher using the same amount of extract after cooking, with the formation of a halo of 25 mm in diameter. Polyphenols of uncooked “Nero di Acerra” beans were inactive against *Ps. aeruginosa.* Such activity was observed after cooking, and 5 µg of such extract produced zones of inhibition of about 9.67 mm. Polyphenols of cooked “Nero di Acerra” were also active against *St.aureus*, at 5 µg. Results seem interesting considering, for example, the fate of polyphenols in the gut and how they can affect the survival of pathogens or probiotics. Further research is underway to understand the influence of individual polyphenols, before and after cooking of beans, on different helpful and pathogen bacteria.
Unknowledgment: The work was supported by "SALVE" and "AGRIGENET" projects, PSR 2007-2013, Campania region

FEMS7-3196
Free Subjects / Other

ANTIMICROBIAL ACTIVITY OF POLYPHENOLS EXTRACTED BY TWO DIFFERENT EVOOS OF THE IRPINIAN PROVINCE, IN SOUTHERN ITALY

F. Nazzaro[1], A. d'Acierno[1], R. Cozzolino[1], A. Martignetti[1], R. Coppola[2], F. Fratianni[1]
[1]Istituto di Scienze dell'Alimentazione - CNR, DiSBA-CNR, Avellino, Italy
[2]DiAAA, DiAAA-University of Molise, Campobasso, Italy

Backgrounds

Extra virgin olive oil (EVOO), the most valuable olive oil category, is greatly appraised around the world for its healthy properties, chiefly due to the presence of monounsaturated fatty acid and antioxidant compounds (polyphenols, tocopherols, etc). In recent times, phenolic compounds present in olive oil stimulated scientific attention due to their beneficial functional and nutritional effects including antioxidant and antimicrobial activities. Production of EVOO represents an important element for the economy of Province of Irpinia in the Campania region, Southern Italy.

Objectives

Our study aimed to evaluate the antimicrobial properties of total polyphenols of EVOOs “Ravece” and “Ruveia”, both cultivated in the village of Montella of Irpinia province.

Methods

Polyphenols from EVOOs "Ravece" and "Ruveia" were obtained following the method of Bayram et al (2012).Total polyphenols were determined (Singleton and Rossi 1965). The antimicrobial activity was evaluated using 4.9 µg of polyphenol extracts of Ravece and Ruveia, as a zone of inhibition, against the human harmful and foodborne pathogens *Bacillus cereus*, *Staphylococcus aureus and Escherichia coli*.

Conclusions

Both extracts were effective in inhibiting the growth of *E. coli* and *B. cereus*. Despite higher content in the total polyphenols, sample "Ruveia" did not give a corresponding greater antimicrobial activity, normalizing the test in terms of GAE polyphenols amount, against the pathogens, respect to "Ravece". However, polyphenols of "Ruveia" but not those of "Ravece" showed activity against the methicillin-resistant strain of *S. aureus*.
Further studies will try to evaluate the influence of individual polyphenols present in the EVOOs on the activity *versus* different pathogens.

References: B. Bayram, et al. Plant Foods Hum Nutr (2012) 67:326–336
VL. Singleton, J.A. Rossi. Am.J. Enol Viticult 16 (1965), 144–158

FEMS7-1877
Free Subjects / Other

INVESTIGATING THE EFFECT OF EFFLUX PUMP INHIBITORS TO CIPROFLOXACIN EFFICACY IN CLINICAL ISOLATES OF ACINETOBACTER SPP.

U. Kayiş[1], S. Ökten[2]
[1]Trakya University Institute of Health Sciences, Department of Basic Pharmaceutical Sciences, Edirne, Turkey
[2]Trakya University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Edirne, Turkey

Backgrounds

Drug resistance has been a great problem for the treatment of infections. The most realistic approach in recent years is researching the inhibition of resistance and enlightening the inhibition mechanisms rather than synthesizing new compounds.

Objectives

In this study, it is aimed to determine i) the effect of PAβN, CCCP and NMP on MIC of ciprofloxacin, ii) to obtain the ideal inhibitor concentration that eliminates the ciprofloxacin resistance.

Methods

Sixty-seven ciprofloxacin resistant *Acinetobacter baumannii* isolated from Trakya University Hospital were included in the study. Ciprofloxacin susceptibility was investigated for these isolates in absence and presence of PAβN, CCCP and NMP. Antimicrobial susceptibility testing was done by microdilution method through the guidelines of CLSI M100-S25. Thirty-two isolates determined to have 4 or more fold decrease in ciprofloxacin MIC values in the presence of the inhibitors, were included in checker board assay.

Conclusions

In the presence of 25mg/L PAßN, CCCP and NMP; 32.83%, 61.50% and 32.30% of the isolates were detected to be susceptible to ciprofloxacin, respectively. In the presence of 100mg/L PAßN, CCCP and NMP; 40.30%, 100% and 61.50% of the isolates were detected to be susceptible to ciprofloxacin, respectively. FIC indexes and inhibitor concentrations that inhibit ciprofloxacin resistance were calculated according to the checker board assay results. The effect of the combinations was reported as synergic or additive.

FEMS7-2374
Free Subjects / Other

THROMBOCIDIN DERIVATIVES AND THEIR ACTIVITY ON THE BACILLUS SUBTILIS CELL ENVELOPE

S. Omardien[1], H. van Veen[2], L. Hamoen[1], J.W. Drijfhout[3], S.A.J. Zaat[4]
[1]University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, The Netherlands
[2]Academic Medical Centre AMC, Central Electron-Microscopy, Amsterdam, The Netherlands
[3]Leiden University Medical Center LUMC, Immunohematology and Blood Transfusion, Leiden, The Netherlands
[4]Academic Medical Centre AMC, Medical Microbiology, Amsterdam, The Netherlands

Backgrounds

Cationic antimicrobial peptides (CAMP) have been proposed as an antibiotic candidate, but presently the mode of action of many antimicrobial peptides to cause bacterial cell death remains abstruse.

Objectives

In this study two CAMPs, TC-19 and TC-84, were characterized by determining their effect on vegetative cells and spores of gram-positive bacteria, using *Bacillus subtilis* strain 168 as a model organism. TC-19 and TC-84 are derivatives of thrombocidins, the major CAMPs of human blood platelets.

Methods

Transcriptomic analysis using microarrays was employed to observe the response of the bacterium to sublethal concentrations. Transmission electron microscopy was used to observe cell envelope deformities. Laurdan was employed to observe changes in membrane fluidity. *Bacillus subtilis* mutant strains expressing proteins fused to the green fluorescent protein (GFP) were examined for delocalization of proteins involved in essential cellular processes. Alexa Fluor® 488 labelled TC-84 was used to evaluate the binding site of this peptide to vegetative cells and spores. Real-time microscopy was employed to determine spore germination or outgrowth inhibition.

Conclusions

The transcriptomic and microscopy results show that TC-19 and TC-84 are bactericidal by targeting the *B. subtilis* cell envelope. The distortion leads to changes in the fluidity of the membrane, causing the delocalization of essential proteins. TC-19 and TC-84 targets the cytoplasmic membrane of germinated spores preventing outgrowth.

FEMS7-2499
Free Subjects / Other

COEVOLUTION BIOMARKERS AND HUMAN SUSCEPTIBILITY TO GASTRIC CANCER IN A POPULATION OF THE COLOMBIAN ANDES

V. Pabon Figueroa[1], A. Angulo[1], J.C. Caguazango[2], A. PAZOS[2]
[1]University of Nariño, Centro de Estudios en Salud CESUN, Pasto, Colombia
[2]University of Nariño, Biology, Pasto, Colombia

Backgrounds

Helicobacter pylori infection is the main risk factor for the development of gastric cancer (GC), however, the prevalence of infection does not predict the incidence of the disease, so it is necessary to understand the host-bacteria relationship as a key factor in gastric etiopathogenesis. In Colombian populations it has been previously identified that the disruption of coevolution of the human-*H.pylori* genome is associated with a greater severity of gastric lesions.

Objectives

To analyze the association between human-*H. pylori* coevolution, human genetic susceptibility to GC and gastric disease in a population of the Andes of Colombia (Túquerres-Nariño).

Methods

We included 82 patients with analysis of human and bacterial ancestry. The genotypes of gene polymorphisms of cytokine IL1β (-511 C/T), IL10 (-819C/T), (-1082 G/A) and TNFα (-308 G/A) were determined by PCR-RFLPs technique and IL1-RN * 2 by standard PCR. We used the logistic regression model to establish the possible association between different biomarkers in the function of gastric lesions.

Conclusions

In the high-risk GC population studied, 70% of patients were carriers of the risk alleles -511T(76%), -819T(81%) and 1082A(54%). This high frequency of risk alleles could predispose individuals as a complementary risk factor to *H. pylori* infection for GC development. The disruption of human-H. pylori coevolution was associated with the presence of atrophic gastric lesions, which could explain the high incidence of GC in the population of the Colombian Andes (OR=5.25, 95% CI: 1.52 -18,10). No association was found between biomarkers of genetic susceptibility to GC (cytokine polymorphisms) and disruption of human-*H. pylori* coevolution.

FEMS7-0800
Free Subjects / Other

PERFORMANCE OF LINE PROBE ASSAY FOR DETECTION OF MDR-TB ON RT-PCR POSITIVE SPUTUM SAMPLES

M.S. Park[1], D.H. KIm[1], Y.M. Kim[1], S.H. Kim[1]
[1]Korea National Institute of Health, TB and respiratory infections, Cheongju, Republic of Korea

Backgrounds

Rapid, accurate diagnosis of drug resistant-TB is critical for timely initiation of treatment and control of disease. Line probe assays (LPAs) were endorsed by the WHO for molecular detection of drug resistance. But application of LPAs to sputum samples is still limited on smear-positive samples.

Objectives

In this study, we evaluate the diagnostic performance of genotype MTBDRplus v1.0 on three commercial RT-PCR kits, GeneXpert MTB/RIF, Anyplex MTB/NTM, and Genedia MTB/NTM, positive samples.

Methods

A total of 678 consecutive specimens were tested with RT-PCR kits and positive samples on either NAATs were subjected to the MTBDRplus. The results of RT-PCR kits were divided into 2 group; low and high bacteria load by CT values and performance characteristic of MTBDRplus was assessed to the MGIT 960 drug susceptibility test.

Conclusions

Of 678 samples, 53.5% MTB isolates were grown in MGIT 960, of which 82.7% were RT-PCR positive, of which 56.5% were interpretable by MTBDRplus. When put the low CT group from RT-PCR as the criteria for application of MTBDRplus, 167 of Xpert, 155 of Seegene, 146 of Genedia positive samples were interpretable by MTBDRplus compared with 127 of smear-positive specimens. The sensitivities of MTBDRplus for the detection of isoniazid and rifampicin resistant from all group were 100%, respectively, and the specificities were comparable across all systems. These findings suggest that put the CT values of RT-PCR as a criteria for application of MTBDRplus, not only accuracy but also affordable sample numbers were increased compared with acid fast staining.

FEMS7-1816
Free Subjects / Other

THE ARCHAEAL DIVERSITY IN HUMAN BODY

M.R. Pausan[1], K. Koskinen[1], M. Beck[2], A.K. Perras[1,2], E. Jantscher-Krenn[3], R. Krause[1], C. Moissl-Eichinger[1]
[1]Medical University of Graz, Internal Medicine, Graz, Austria
[2]University of Regensburg, Microbiology and Archaea, Regensburg, Germany
[3]Medical University of Graz, Obstetrics and Gynecology, Graz, Austria

Backgrounds

Trillions of microbes live in and on the human body, forming the human microbiota. These complex communities contain taxa from all three domains of life (Bacteria, Eukarya and Archaea) as well as viruses. These microorganisms function like an “invisible organ” by helping in energy harvest and storage, contributing to metabolic functions, protecting humans against pathogens, and educating the immune system. Most studies target the predominant bacterial community of this “invisible organ” and the (so far detected) not-pathogenic archaeal community and its diversity remains largely hidden. Since archaea have been associated mainly with the human gut and oral microbiome, little is known about the presence and diversity of archaea in other body sites.

Objectives

In this study, we aim to explore the archaeal diversity and presence in different body sites of the human body (e.g. nose, lung, vagina, appendix, and oral).

Methods

The study is based on a NGS approach, by amplifying the archaeal 16S rRNA with specific primers and analyzing the results through dada2 pipeline and R.

Conclusions

The archaeal diversity within the human body is higher than it was expected and is not only composed of archaea from Euryarchaeota phylum, but also Thaumarchaeota and Woesearchaeota. Furthermore, the methanogenic archaea that are mainly associated with the gut and oral microbiome are rather present in other body sites as well like the human nose and in the lung. This indicates that the archaea as bacteria are present in different body sites and are not restricted to a specific body site.

FEMS7-0412
Free Subjects / Other

DESIGN, SYNTHESIS AND IN VITRO AND IN VIVO ACTIVITY OF FUROXAN DERIVATIVES AGAINST MYCOBACTERIUM TUBERCULOSIS

F. Pavan[1], P. Souza[2], L. Marino[2], C. Ribeiro[2], G. Fernades[2], M.C. Chung[2], R. Fruttero[3], J. Santos[2]
[1], Araraquara, Brazil
[2]UNESP, Biological Sciences, Araraquara, Brazil
[3]Università degli Studi di Torino, 3Dipartimento di Scienza e Tecnologia del Farmaco, Turin, Italy

Backgrounds

Tuberculosis (TB) remains a serious health problem responsible to cause millions of deaths annually. As many others infectious diseases, no or few drugs were developed after the gold age (until 60´s).

Objectives

Design, synthesize and evaluate *in vitro* and *in vivo* a novel series of hybrid furoxan derivatives against *Mycobacterium tuberculosis* (Mtb).

Methods

In vitro – Determination of minimal inhibitory concentration (MIC_{90}) against clinical isolates (susceptible and resistant) was performed by Resazurin Microtiter Assay (REMA) and cytotoxicity assay (IC_{50}) on MRC-5 and J774A.1 cells; activity against non-replicant bacteria by LORA (Low Oxygen Recovery Assay); mutagenicity was performed by AMES test; time-kill curve was performed by 20 days of incubation; stability study was performed by HPLC and finally, microarray to determine the mechanism of action. *In vivo* – Oral bioavailabity for pharmacokinetic (PK) screening and infection/treatment were performed by BALB/c mice.

Conclusions

A library of forty compounds were synthesized and tested against Mtb and cytotoxicity. Of those, we chose the best compound to proceed further studies, the compound 14c ((E)-4-(4-((2-isonicotinoylhydrazono)methyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide. 14c compound showed good activity against non-replicant bacteria and all clinical resistant isolates. Not mutagenic. Bactericidal pharmacodinamic. Showed to be stable at pH 7.0 and 5.0 and seems to inhibit protein synthesis as mechanism of action. *In vivo* showed good PK and after 20 days of treatment with 200 mg/kg of body weigh sterilizing effect. Based on these results we are performing others further studies but we are able to conclude 14c as promising new class against TB.

FEMS7-1435
Free Subjects / Other

REPURPOSING CLINICALLY APPROVED CEPHALOSPORINS FOR TUBERCULOSIS THERAPY

S. Ramon Garcia[1], R. González del Río[2], A. Santos Villarejo[2], G. Sweet[3], F. Cunningham[2], D. Barros[2], L. Ballell[2], A. Mendoza-Losana[2], S. Ferrer-Bazaga[2], C. Thompson[3]
[1]University of Zaragoza, Department of Microbiology, Zaragoza, Spain
[2]GlaxoSmithKline - Diseases of the Developing World, TB DPU, Tres Cantos, Spain
[3]University of British Columbia, Department of Microbiology, Vancouver, Canada

Backgrounds

While modern cephalosporins developed for broad spectrum antibacterial activities have never been pursued for tuberculosis (TB) therapy, we explored their potential repositioning as new anti-TB drugs.

Objectives

We previously demonstrated that combinatorial drug therapy might be employed to increase the efficacies of available antibiotics, allowing them to be repurposed for TB therapy within synergistic combinations [doi: 10.1128/AAC.00474-11]. Rifampicin, the cornerstone drug for TB therapy, is not administered at its optimal clinical dose due to long-established toxicity concerns. If the anti-mycobacterial activity of rifampicin could be increased, TB therapy could be shortened, thus reducing the rate of transmission and the emergence of drug resistance.

Methods

We screened an in-house library of ca. 600 commercially available antibiotics, and found that (i) cephalosporins had strong synergies with rifampicin and ethambutol, a first-line anti-TB drug (4- to 64-fold more active in combination than either drug alone); (ii) common chemical patterns required for single drug activity against *Mtb* were identified using structure-activity relationships (SAR) studies; (iii) synergy was observed even under intracellular growth conditions where beta-lactams typically have limited activities; (iv) cephalosporins and rifampicin were synergistic but limited synergy was observed with rifapentine or rifabutin; (v) Clavulanate was a key synergistic partner in triple combinations, together with cephalosporins rescued the activity of rifampicin against a rifampicin resistant strain; (vi) uptake experiments demonstrated that synergy was not due exclusively to increased rifampicin accumulation within the mycobacterial cells, and; (vii) cephalosporins were also synergistic with new anti-TB drugs such as bedaquiline and delamanid.

Conclusions

We identified first generation cephalosporins having clinically relevant inhibitory concentrations, both alone and in synergistic drug combinations. Cephalosporins are orally bioavailable with good safety profiles; together with their anti-mycobacterial activities reported here, it suggests that they could be repurposed within new combinatorial TB therapies.

For more info see here: doi:10.1038/srep34293.

FEMS7-0395
Free Subjects / Other

ASSESSMENT OF NULL MUTATIONS IN GSTT1 AND GSTM1, TLR 2 POLYMORPHISM (ARG677TRP) AND EFFICACY OF COMMONLY PREVAILING ERADICATION THERAPIES IN HELICOBACTER PYLORI INFECTED PATIENTS OF PAKISTAN

F. Rashid[1], H. Noor[2], M. Ali[2], R. Kamran[3], A. Yameen[4], T. Ahmad[4], F. Rasheed[5]
[1], Islamabad, Pakistan
[2]Pakistan Institute of Medical Sciences, Department of Gastroenterology, Islamabad, Pakistan
[3]Pakistan Institute of Medical Sciences, Microbiology, Islamabad, Pakistan
[4]Pakistan Institute of Nuclear Science and Technology, Delta V Mass Spectrometer Lab, Islamabad, Pakistan
[5]Nuclear Medicine Oncology & Radiotherapy Institute, BreathMAT Lab, Islamabad, Pakistan

Backgrounds
The prevalence of H. pylori, its associated risk factors and efficacy of eradication therapies against it, varies widely in different geographical regions. In the developing countries H. pylori infection is more prevalent. Therefore, assessment of efficacy of prevailing eradication therapies as well as mutations is essential to decrease the epidemiology. Up till now, no study has been carried out to determine the association of TLR 2 (Arg677Trp) polymorphism as well as GSTT1 and GSTM1 polymorphism with H. pylori and gastro-duodenal diseases as genetic risk factor.
Objectives
To determine the prevalence and association of TLR-2 and GST genes polymorphism with H. Pylori infection along with efficacy and side effects of prevailing treatment therapies in the local population suffering from gastro-duodenal diseases.
Methods
A total of 159 patients were enrolled to determine the prevalence and effectiveness of first line eradication therapies namely standard triple therapy and sequential therapy as well as second line eradication therapies which included levofloxacin based triple therapy and moxifloxacin based triple therapy. The status of H. pylori infection in patients was determined by 13C Urea Breath Test. Also, 130 individuals (59 H. pylori infected and 71 H. pylori non-infected) were enrolled separately for PCR based detection of polymorphism in TLR-2 Arg677Trp (C2029T), GSST-1 and GSTM1 genes.
Results
The prevalence of H. pylori was found 72.3% in symptomatic population. The eradication rates for standard triple therapy, sequential therapy levofloxacin based therapy and moxifloxacin based therapy were 66.7%, 62.9%, 30.8% and 14.3% respectively. No association was found between SNP in TLR-2 Arg677Trp (C2029T) and null mutations in GSST-1 genes with H. pylori infection ($p>0.05$).
Conclusions
The prevalence of H. pylori infection was very high in Pakistani population with gastro-duodenal diseases. The resistance to prevailing first line and second line eradication therapies was also found very high in our region. Furthermore, gastro-duodenal disorders were not found associated with GSTM1 and GSTT1 null mutations as well as TLR-2 (Arg677Trp) polymorphism.

FEMS7-3041
Free Subjects / Other

THE EFFECT OF RUMEN BACTERIA FROM SHEEP ADAPTED TO A TANNINIFEROUS DIET ON IN-VITRO FERMENTATION PARAMETERS OF PISTACHIO HULL BY-PRODUCT USING BOVINE INOCULUM

Y. Babaei[1], Y. Rouzbehan[1], D. Alipour[2]
[1]Tarbiat Modares University, Department of Animal Science, Tehran, Iran
[2]Faculty of Agriculture- Bu-Ali Sina University-, Department of Animal Science, Hamedan, Iran

Backgrounds

Backgrounds: Drought climatic conditions have led to a scarcity of ruminant feeds in many countries. The use of agricultural by-products such as pistachio hulls (PH) could be an alternative to conventional feeds in overcoming this problem. The major limitation of using these by-products is the presence of high levels of tannins which have a negative effect on ruminal fermentation parameters. Mature Taleshi sheep that consume tannin rich feeds such as oak leaves appear to have ruminal bacteria with defensive mechanisms against these polyphenols.

Objectives

Objectives: The capability of ruminal isolated tannin degrading bacteria from these animals (i.e., Taleshi sheep) to ferment a tanniniferous by-products (i.e., pistachio hulls) incubated with rumen fluid from Holstein dairy cows was assessed. Six Gram positive cocci were isolated from the rumen of the Taleshi sheep and their 16s rRNA gene sequences showed them to be closely related to *Streptococcus gallolyticus*.

Methods

Methods: In three runs of in vitro gas production (GP), the effect of two of these isolates incubated with buffered ruminal fluid of Holstein cow and PH was evaluated. The GP was recorded from 1 to 96 hours of incubation. Incubating either of the isolates with PH caused a significantly higher in vitro gas production, in vitro organic matter disappearance, metabolisable energy and volatile fatty acids than those without isolate.

Conclusions

Conclusions: The improvement in the ruminal parameters when either of the isolates was used suggested the possible presence of isolated tannin-degrading bacteria (*Streptococcus gallolyticus* sp.).

FEMS7-0310
Free Subjects / Other

IN VITRO ANTILEISHMANIAL ACTIVITY OF SATUREJA KHUZESTANICA JAMZAD AND HERACLEUM PERSICUM DESF. EX FISCH ON LEISHMANIA PROMASTIGOTES USING MTT ASSAY

B. Sadeghi-Nejad[1], S. Khademvatan[2], A. Eskandari[3], S. Yusef Naanaie[4]
[1]Abadan School of Medical Sciences- Abadan- Iran, Myco-Parasitology, Ahvaz, Iran
[2]Urmia University of Medical Sciences- Urmia- Iran, Myco-Parasitology-, Urmia, Iran
[3]Faculty of Pharmacy- Ahvaz Jundishapur University of Medical Sciences- Ahvaz- Iran, Medicinal Chemistry-, Ahvaz, Iran
[4]Agricultural and Natural Resources Center- Ahvaz- Iran, Agricultural and Natural Resources Center, Ahvaz, Iran

Backgrounds

Leishmaniasis is a major health problem global and affects millions of people especially in developing countries. Science, there is no immunoprophylaxis (vaccination) accessible for Leishmania infections and commercial drugs are unsatisfactory. .

Objectives

The objective of the present survey was to state the antileishmanial activity of two herbal medicine (*Satureja khuzestanica* leaf and *Heracleum persicum* fruit) extracts were evaluated against *Leishmania major* and *Leishmania infantum* using colorimetric MTT (2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide) assay and compared to the Glucantime as a reference.

Methods

The leaves extracts of selected plants were obtained by maceration. The *in vitro* assays were carried out on *Leishmania major* and *Leishmania infantum* using colorimetric MTT assay in comparison with Glucantime. The concentration-response curves tested extracts and glucantime solutions were designed and IC_{50} values were located.

Conclusions

Anti-Leishmina effects of *Satureja khuzestanica* and *Heracleum persicum* on *L. major* and *L. infantum* promastigote were revealed with 50% inhibitory concentration (IC50) values of 4.8 and 7.5 mg ml^{-1} for *Satureja khuzestanica*, 29.3 and 14.7 mg ml^{-1} for *Heracleum persicum*. In the comparison with the standard drug, glucantime, which had IC50 value of 40.2 mg ml^{-1} for *L.major* and 18.5 mg ml^{-1} for *L. infantum* promastigote after 72 hours incubation, respectively. ***Conclusions:*** These results revealed that compounds from *Satureja khuzestanica* and *Heracleum persicum* have anti-leishmania properties that necessary to survey the effects of these extracts on leishmania genus in animal models in future.
Keywords: Antileishmanial activity, *Leishmania major*, *Leishmania infantum*, Glucantime, Promastigote, MTT assay.

FEMS7-0644
Free Subjects / Other

SELECTION OF BACTERIA FOR INDUCING TOLERANCE TO SALINITY AND TEMPERATURE STRESS IN TOMATO PLANTS

M.K. Sang[1], S.J. Yoo[1], J. Song[1], H.Y. Weon[1]
[1]National Academy of Agricultural Science, Agricultural Microbiology, Wanju-gun, Republic of Korea

Backgrounds

Abiotic stress such as salinity and temperature stresses are severe environmental constraint to agricultural productivity. Plant growth-promoting rhizobacteria(PGPR) could play an important role in alleviation of abiotic stress as well as biotic stress in plants.

Objectives

We investigated effects of bacterial strains on inducing tolerance to abiotic stress including salinity, high and low temperature in tomato plants.

Methods

To do this, we isolated 1,944 bacterial strains from rhizo- and endosphere of tomato plants in various regions in South Korea, and tested bacterial characters related to plant growth promotion including production of phosphate solubilization and indole-acetic acid (IAA), and activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase; bacterial adaptability to salinity(-70, -500, and -1000kPa salt solution) and temperature conditions(10, 25, and 40^{o}C). Through these in vitro assays, we pre-screened 46 strains, sequentially, tested plant trials in a greenhouse.

Conclusions

After challenging salinity or temperature stress to plants treated with bacterial suspension, we evaluated plant growth and selected four strains as potential agents to help plant tolerate abiotic stress including both high salinity and high/low temperature stress. We will investigate how the strains could interact with plants and alleviate abiotic stress by induced tolerance in tomato plants.

FEMS7-1948
Free Subjects / Other

AEROGELS OF ENZYMATICALLY OXIDIZED GALACTOMANNANS FROM LEGUMINOUS PLANTS: VERSATILE ANTIMICROBIAL RELEASE SYSTEMS

T. Silvetti[1], P. Campia[2], L. Merlini[3], M. Brasca[1], Y. Galante[3]
[1]Institute of Sciences of Food Production ISPA-CNR, Italian National Research Council CNR, Milan, Italy
[2]Department of Agricultural and Environmental Sciences Production- Landscape- Agroenergy, University of Milan, Milan, Italy
[3]Institute of Chemistry of Molecular Recognition ICRM, Italian National Research Council CNR, Milan, Italy

Backgrounds

Thanks to their biodegradability and biocompatibility and their porous structure, polysaccharides nanostructured aerogels provide high added-value materials suitable to be loaded with active principles. In this study, new aerogels were obtained from enzymatic-oxidization of galactomannans (GM) extracted from the leguminous plants fenugreek, sesbania and guar. TEMPO-mediated, laccase oxidation of GM in aqueous solution caused a viscosity increase up to fifteen-fold, generating elastic and stable hydrogels, which, upon lyophilization, were converted into water-insoluble aerogels, capable of uptaking water or solvents several times their own original weight.

Objectives

The purpose of this work was to evaluate the efficiency of GM-based aerogels for the release of antimicrobial compounds in active form.

Methods

Antimicrobial actives used in the food (nisin, lysozyme), medical (polymyxin B) and industrial (5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (CIT/MIT)) fields were absorbed into the aerogels from aqueous solutions. Repeated water rinsing, dry blotting and re-lyophilization of the hydrogels were performed to generate “loaded” aerogels. The release of the incorporated actives against *Pseudomonas aeruginosa, Serratia marcescens, Escherichia coli, Salmonella* Typhimurium, *Halvia alvei* and *Enterobacter cloacae* (polymyxin B) and *P. aeruginosa, E. coli, Staphylococcus aureus*, *Saccharomyces* spp., *Pichia* spp. and *Penicillium* spp. (CIT/MIT), *Clostridium tyrobutyricum* and *Enterococcus faecalis* (nisin), and *C. tyrobutyricum* (lysozyme) was monitored through biochemical, microbiological and spectrophotometric assays.

Conclusions

The actives released from GM-aerogels were able to control microbial growth *in vitro*, even in the presence of a relatively high cell concentration thus these biomaterials might represent innovative and versatile and sustainable carrier systems of active principles for food, biomedical and industrial applications.

FEMS7-1928
Free Subjects / Other

ABCS OF A NOVEL ANTIMICROBIAL AGENT: ANTIBACTERIAL ACTIVITY, BACTERIAL "BACKTALK" AND COMPARATIVE CYTOTOXICITY

L. Tonoyan[1], G.T.A. Fleming[1], P.H. Mc Cay[1], V. O'Flaherty[1]
[1]National University of Ireland- Galway, Microbiology, Galway, Ireland

Backgrounds

In the context of growing antibiotic resistance, alternative therapies are urgently required. Peroxidase-catalysed systems produce effective antimicrobial agents, but have drawbacks associated with enzyme stability. Here, we report a novel and highly efficient biocidal complex (BC), generated by enzyme-free reaction between hydrogen peroxide and two oxidizable substrates (iodide and thiocyanate).

Objectives

This study aimed to evaluate the antibacterial properties, the potential for induction of resistance and cross-resistance, and to investigate the potential cytotoxicity of BC.

Methods

Micro-dilution and time-kill assays were used to determine minimum inhibitory, bactericidal concentrations and killing kinetics of BC. Mono- and dual-species biofilms were established in modified Robbin's devices and the biofilm eradication concentrations of BC were defined. Multi-passage studies were performed in the presence of sub-inhibitory concentrations of BC to select for resistance, whereas, chemostats were used to investigate bacterial adaptation to BC and resistance to levofloxacin under nutrient limiting environment. MTT, haemoglobin release and comet assays were used for the evaluation of cytotoxicity, haemolytic activity and genotoxicity.

Conclusions

BC was a potent bactericidal agent, causing rapid death of pathogenic bacteria in planktonic and biofilm forms. Bacteria exposed to BC failed to develop resistance after serial passages. Furthermore, chemostats operated with BC-selection pressure yielded population with no altered susceptibility towards BC and levofloxacin, revealing that BC usage was not promoting tolerance to itself and cross-resistance to antibiotics. Cytotoxicity studies indicated non-selective cytotoxic (yet biocompatible), dose-dependent haemolytic and non-genotoxic profile of BC. Thus, it may be used to treat infection sites and to decontaminate surfaces.

FEMS7-0718
Free Subjects / Other

THE PESTICIDE BEHAVIOR AND AZOLE RESISTANCE DEVELOPMENT IN RICE PADDY FIELDS

A. Vaezi[1], *H. Badali*[1], *H. Fakhim*[1]
[1]*mazandaran university of medical sciences, Medical Mycology and Parasitology, sari, Iran*

Backgrounds

Trend of azole resistance in *Aspergillus fumigatus* in different continents is important to conduct surveillance studies to prevent and control aspergillosis and antifungal stewardship. Tricyclazole is an agrochemical fungicide belonging to the class of triazole, commonly used in paddy rice fields in Asian countries which inhibits the pentaketide (DHN) and branch pathways of melanin synthesis in *Pyricularia oryzae*. Although emerging azole resistance contributes to treatment failures is unknown, triazole fungicide use in agriculture has been linked to this phenomenon in Europe.

Objectives

We aimed to study the current status of azole resistance *A. fumigatus* obtained from paddy fields with exposure to tricyclazole.

Methods

A total of 108 soil sampling were collected from four different locations of paddy fields in Mazandaran, 31 (28.7 %) of soil samples harbored *A. fumigatus*. 11 of 31 *A. fumigatus* isolates grew on SDA supplemented with itraconazole or voriconazole at 48 °C. The isolates were confirmed by partial sequencing of the β-tubulin gene. In vitro antifungal susceptibility tests of 11 isolates were performed against seven antifungal agents based on CLSI guideline (M38-A2).

Conclusions

Posaconazole and voriconazole were 2 log ^{2}-dilution steps and 3 log ^{2}-dilution steps less active than luliconazole and lanoconazole, respectively. The highest MICs in increasing order were constantly found with luliconazole, lanoconazole, posaconazole, caspofungin and amphotericin B. Only two *A. fumigatus* isolates harboured TR34/L98H variant. In contrast, TR46/Y121F/T289A and other point mutations were not detected in the *CYP51A* promoter region. In this study, one might conclude that tricyclazole, with different mechanism of action against medical azoles, induce azole resistance in *A. fumigatus* isolates. Further knowledge about the role of tricyclazole on the azole resistance development is required, in order to avoid a highly dangerous pesticide selection pressure that would enhance the risk of azole resistance.

FEMS7-2269
Free Subjects / Other

POTENTIAL OF USING HISTIDINE KINASE INHIBITORS AGAINST COLISTIN-RESISTANT KLEBSIELLA PNEUMONIAE

N. Velikova[1]
[1]*Wageningen University, Animal Sciences, Wageningen, Netherlands Antilles*

Backgrounds

Antimicrobial resistance in general, and colistin resistance in particular, requires the urgent development of novel approaches to fight resistant pathogens. Bacterial two-component systems have been linked to colistin resistance in *Klebsiella pneumoniae*. Here, we report the potential to use recently discovered bacterial histidine kinase inhibitors against colistin-resistant *K. pneumoniae* and the other ESKAPE pathogens.

Objectives

The objectives of the presented work were to study the synergistic effect of histidine kinase inhibitors and colistin *in vitro* and *in vivo*.

Methods

Microdilution assays to determine fractional inhibitory concentrations
G. mellonella larvae as an infection model to study the synergistic effect in vivo

Conclusions

Research in progress

FEMS7-2181
Free Subjects / Other

MOLECULAR CHARACTERIZATION FOR ESBL GENES OF E.COLI ISOLATES FROM LIVESTOCK ANIMALS IN ITALY REVEALS HIGH PREVALENCE OF TEM AND LOW PREVALENCE OF CTX-MI GENES

S. Migliore[1], B. Amato[2], M. La Giglia[1], C. Piraino[3], M. Goria[4], V. Di Marco Lo Presti[2], M. Vitale[5]
[1]Istituto Zooprofilattico Sperimentale della Sicilia, Molecular Biology, Palermo, Italy
[2]Istituto Zooprofilattico Sperimentale della Sicilia, Area barcellona P.G., Palermo, Italy
[3]Istituto Zooprofilattico Sperimentale della Sicilia, Diagnostica Specialistica, Palermo, Italy
[4]Istituto Zooprofilattico Sperimentale of Piedmont, Molecular Biology, Turin, Italy
[5]Zooprofilattico Sperimentale della Sicilia, Molecular Biology, Palermo, Italy

Backgrounds

Pathogenic strains of *Escherichia coli* (*E. coli*) are classified as: enterotoxigenic *E. coli* (ETEC); enteroinvasive (EIEC), enteropathogenic (EPEC) and enterohemorrhagic (EHEC). These groups differ in their epidemiology and pathogenesis and their O:H serotypes. The presence and diffusion of antibiotic resistance such as extended spectrum of beta lactamases (ESBL) is a growing problem for several gram-negative bacteria. The first plasmid-mediated ß-lactamase was described in the early 1960s from a single strain of *E. coli* isolated from a patient named Temoniera in Greece (TEM). TEM-1 gene is now found in many different species of the family *Enterobacteriaceae*

Objectives

The aim of the study was the analysis of virulent genes and ESBL genes in *E .coli* strains isolated from livestock animals. A total of 36 isolates from cattle and pigs were analyzed.

Methods

Genetic analysis were performed by multiplex PCRs. Two PCRs target the genes *aggR, aap, aatA, astA, pet, shf , irp2, set1A* and *eae.* Other two Multiplex PCRs target genes encoding ß-lactamases: TEM, OXA, SHV, CTX-M, CMY and DHA type β-lactamases.

Conclusions

On a total of 30 *E.coli* isolates from livestock 20 resulted positive for TEM only. 5 resulted positive for both TEM and CTX- MI and 11 were completely negative for ESBL genes. Interestingly the different prevalence was probably related to farm management since higher prevalence of virulence and ESBL genes was present in intensive farming in contrast to extensive and traditional farm management.

Acknowledgement: The research is supported by grant CCM2015AMR of Italian Ministry of Health

FEMS7-1810
Free Subjects / Other

EFFICIENT REMOVAL OF BISPHENOL A AND DICLOFENAC BY LACCASE FROM PLEUROTUS OSTREATUS

K. Wlizło[1], *J. Polak*[1], *A. Jarosz-Wilkołazka*[1]
[1]*Maria Curie-Skłodowska University, Department of Biochemistry, Lublin, Poland*

Backgrounds

The problem encounters by the modern biotechnology is the bioremediation of wastewaters contaminated by commonly used compounds, such as bisphenol A (BPA), used in the plastic industry, and diclofenac (DCF), popular anti-inflammatory pharmaceutical. Considering an endocrine disrupting activity of BPA and a negative effect of DCF on water organisms, the degradation of those compounds has become an important and extensively studied issue. There are some papers indicating the possibility of BPA and DCF degradation by microorganisms such as *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa* or ammonia oxidizing bacteria, however, the application of ligninolytic fungal enzymes such as laccase may provide even higher efficiency of this process.

Objectives

The aim of presented work was to determine the ability of laccase from *Pleurotus ostreatus* to the degradation of bisphenol A and diclofenac by the enzyme with or without addition of specific mediators increasing the efficiency of the reaction.

Methods

Purified laccase from *P. ostreatus* was incubated with BPA or DCF with or without addition of TEMPO or ABTS used as mediator. The transformation process was analyzed using high performance liquid chromatography (HPLC).

Conclusions

Laccase from *P. ostreatus* was an efficient biocatalyst during BPA non-mediated degradation, whereas degradation of DCF was more efficient in the presence of mediators. However, the addition of mediators had an impact on different products formation during degradation process, which will be further studied by the ecotoxicity evaluation using *Vibrio fisheri*.

This work was partially supported by National Science Centre (2015/17/N/NZ9/03647).

FEMS7-0961
Free Subjects / Other

IDENTIFICATION OF ANTIBACTERIAL COMPOUNDS IN PROPOLIS AND MECHANISTIC INSIGHT INTO ITS BACTERICIDAL ACTION

Y. Yoshimasu[1,2], T. Ikeda[3], N. Sakai[4], A. Yagi[4], Y. Morinaga[2], S. Furukawa[2], R. Nakao[1]
[1]National Institute of Infectious Diseases, Bacteriology I, Shinjuku, Japan
[2]Nihon University, Food Bioscience and Biotechnology, Fujisawa, Japan
[3]Sojo University, Pharmaceutical Sciences, Kumamoto, Japan
[4]Olympus Corporation,
Micro System Technology Department- Medical Elemental Technology Development Division, Hachioji, Japan

Backgrounds

Propolis, a bee product, exhibits biological activities including antimicrobial action, and has been widely used as a folk medicine against infectious diseases. However, the reason for propolis's prophylactic/therapeutic potential against infectious diseases remains obscure.

Objectives

We sought to reveal the mechanism by which propolis attacks pathogen, and identify antibacterial compounds in propolis.

Methods

The antibacterial mechanism of propolis against *Porphyromonas gingivalis* or *Escherichia coli* was investigated by comprehensive approaches, including spatial/temporal analysis using high-speed atomic force microscopy (HS-AFM) with nanometer resolution, and a fluorescence-based membrane potential assay using flow cytometry. Antibacterial compounds were isolated from ethanol-extracted propolis. Fractions from the purification were subjected to a growth inhibition assay using *P. gingivalis*, to assess their antibacterial activities.

Conclusions

HS-AFM analysis showed that propolis immediately triggered development of aberrant membrane blebs, followed by bleb-bleb fusion events on the cell surface of *P. gingivalis*. We found that propolis induced biphasic membrane perturbation against *E. coli* cells, i.e., low propolis concentrations depolarized membranes and high propolis concentrations hyperpolarized membranes and led to loss of membrane integrity. Furthermore, we identified at least three antibacterial compounds in propolis with low cytotoxicity to human oral epithelial cells. It is notable that a pentacyclic triterpenoid of plant origin, ursolic acid, showed the highest lipophilic properties and strongest antibacterial effect (MIC: 31.25 μM) of the three antibacterial compounds identified in the present study. We therefore suggest that propolis is an exquisite membrane-targeting antibacterial agent with prophylactic/therapeutic applicability against periodontitis and other bacterial infectious diseases.

FEMS7-1733
New Methods and Techniques

A NEW METHOD OF DNA ISOLATION FROM SPUTUM SAMPLES FOR DIAGNOSIS OF TUBERCULOSIS WITHOUT ANY LABORATORY INSTRUMENTS

E. Ağel[1], H. Sağcan[2]
[1]TUBİTAK Marmara Research Center, Food Institute, Kocaeli, Turkey
[2]Istanbul University, Molecular Biology and Genetic, Istanbul, Turkey

Backgrounds

DNA isolation from difficult specimens is an important point in the detection of many pathogens and bacteria. Today, many DNA isolation methods have complex processing steps and require laboratory devices. Therefore, a simply DNA isolation method that can be used not only in laboratory but also in the field is required.

Objectives

The aim of this study is development of a simple and effective method for homogenization of sputum samples and isolation of DNA without any laboratory instruments.

Methods

Homogenization was carried out in 5% trypsin solution at room temperature for 30 min. Before DNA isolation, samples were contaminated with 10^1 to 10^5 cfu/mL *M. tuberculosis.* After homogenization, samples were passed through the silica solid phase column. Before using, columns were conditioned with chemical treatment in order to better retention of DNA. After passing sample through the column, column was washed with 80% isopropyl alcohol(10 mL). In order to remove alcohol, column was dried. Finally, DNA in the column was eluted with 10 mM TE solution(2 mL).

Conclusions

Trypsin homogenization method is found more successful and simpler than other methods in the previous studies. Studying in room temperature is the additional advantage of this method.

Five different DNA isolation methods have been compared and it has been reported that QIAGEN Qiamp is the most successful method. In the developed new method, 10^2 cfu/mL concentrations can be detected, so the method is as successful as the Qiagen. The most important point of this method is isolation of DNA without using any complex devices.

FEMS7-0039
New Methods and Techniques

CAPILLARY CYTOMETRY, A NEW APPROACH TO VALIDATE THE ANTIMICROBIAL EFFICIENCY OF ISOLATOR DISINFECTION.

G. CARRE[1], C. MULLER[2], J.P. GIES[1], J. DUBOIS[3], P. URRUTTI[4], F. GUERRIN[3], P. ANDRE[1]
[1]Université de Strasbourg, Laboratoire de Biophotonique et pharmacologie UMR 7213, ILLKIRCH, France
[2]Université de Strasbourg, Institut Pluridisciplinaire Hubert Curien- UMR 7178, Strasbourg, France
[3]Groupe ARECO, Innovation, GRASSE, France
[4]OSMOSE, Recherche et développement, ROQUEFORT LES PINS, France

Backgrounds

In the pharmaceutical industry, the use of isolator technology increases in a significant way. Hydrogen peroxide vapor (HPV) is the most commonly gas used to disinfect isolators.

Objectives

The disinfection efficiency is determined by biological indicators (BIs) *i.e.* a coupon inoculated with 10^6 spores of *Geobacillus stearothermophilus*. According to the ISO 14161 norm, a 7 days period of incubation at 56°C in tryptic soy broth is recommended before declaring a BI positive (incomplete spore disinfection) or negative (complete spore inactivation).

To determine more rapidly this effectiveness, a capillary cytometry technic was optimized.**Methods**

Membrane integrity, regarded as a reflection of cell viability, is monitored by a live/dead kit (Invitrogen, L13152). By setting an arbitrary threshold to 2.10^5 events/mL, it is possible to predict 24hrs later, with a 100% efficiency, that the broth medium will be positive (cloudy) 7 days later. In the same manner a value under 4.10^4 events/mL will predict over 98% that the broth will remain sterile. If the value is between, a further incubation of 24hrs is required and a 48hrs later value over 2.10^5 events/mL, indicates that the broth medium will be positive (cloudy) 7 days later.

Conclusions

Thus, thanks to our original capillary cytometry technic, we now can determine the presence/absence of surviving cells and thereby determine the efficiency of isolator disinfection in a 24-48hrs time period instead of 7 days.

FEMS7-1316
New Methods and Techniques

NEW TOOLS AND DATA PROCESSING PROCEDURES FOR QUICK METAPROTEOMICS

J. Armengaud[1], M. Charlotte[1], M. Guylaine[1], S. Gérard[1], A.B. Béatrice[1], P. Olivier[1]
[1]CEA, DRF-ibitecS-SPI-Li2D, Bagnols-sur-Cèze, France

Backgrounds

Metaproteomics allows i) establishing an inventory of organisms present in a sample, even for a complex microbiota, and ii) documenting new insights into functional pathways and interactions between microorganisms. Combining this approach with metagenomics is crucial for in-depth exploration of microbiomes.

Objectives

Our long-term goal is simplifying metaproteomics data acquisition and data treatment for being able to explore any microbiota in a few hours even without sequencing data. Here, we propose a lab-assembled microbiota reference for evaluating data processing procedures for metaproteomics. We also designed a new bioinformatics pipeline for quick analysis of samples.

Methods

We assembled an artificial microbiota reference comprising 24 bacterial species covering 20 genera and 5 phyla. The proteins from this standard were extracted and tryptic peptides were generated. We acquired several metaproteomics dataset by recording high quality tandem mass spectra on this peptide pool using a tandem mass spectrometer incorporating an ultra-high-field orbitrap analyzer. Typically, a set of 117,703 spectra have been recorded over a 3h run, allowing identification and characterization of the 24 bacteria. Important functional information can be extracted from these data with the detection of 4,371 candidate proteins. Furthermore, we exemplified the possible applications of this procedure to decipher the microbiota (bacteria, archaea, fungi, and microalgae) of diverse environmental samples: wall deposits, biofilms, soils, and feces.

Conclusions

Based on this reference dataset, we propose an innovative data processing procedure for minimizing false-positive identification of organisms, and performing species-resolved metaproteomics with the most appropriate pan-proteomics database.

FEMS7-0409
New Methods and Techniques

MULTILOCUS VARIABLE-NUMBER TANDEM REPEAT TYPING OF MYCOBACTERIUM KANSASII

Z. Bakula[1], A. Brzostek[2], A. Podpora[1], P. Parniewski[2], J. Dziadek[2], T. Jagielski[1]
[1]University of Warsaw- Faculty of Biology,
Department of Applied Microbiology- Institute of Microbiology, Warsaw, Poland
[2]Polish Academy of Sciences,
Mycobacterium Genetics and Physiology Unit- Institute of Medical Biology, Łódź, Poland

Backgrounds

Mycobacterium kansasii is one of the most virulent nontuberculous mycobacteria (NTM). It is also one of the six most commonly isolated NTM species around the world. The observed genetic homogeneity of *M. kansasii* strains results in poorly understood epidemiology of infections due to this pathogen.

Objectives

The aim of this study was to search the genome of *M. kansasii* for tandem repeat sequences, similar to variable number tandem repeat (VNTR) sequences in *M. tuberculosis* allowing for intraspecies differentiation/species fingerprinting.

Methods

The *M. kansasii* genome (GenBank, NCBI, Reference Sequence: NC_022663.1) was screened for the occurrence of TR loci with the Vector NTI Software (Thermo Fisher Scientific, Waltham, MA, USA). Primers were designed to anneal to the flanking region of TRs utilizing same software as for the TR screening. PCR protocols were tested on a collection of 15 strains representing six *M. kansasii* subtypes (I-VI) as well as 90 *M. kansasii* clinical strains isolated from Polish respiratory patients between 2000 and 2015.

Conclusions

A total of 17 potential VNTR loci with high variability in the number of TRs were identified among strains of *M. kansasii* subtypes. Additionally, six of the tested VNTRs (1, 2, 7, 15, 17, 20) showed variation among *M. kansasii* clinical strains (all of type I). In summary, the newly designed VNTR-based typing method of *M. kansasii* appears to be a promising tool for *M. kansasii* fingerprinting and thus may help to better explore the transmission routes of *M. kansasii* infections.

FEMS7-2521
New Methods and Techniques

NOVEL PHYLOGENETIC MARKERS FOR COST-EFFICIENT AND ACCURATE TYPING OF PROTOTHECA MICROALGAE

Z. Bakula[1], Ś. Marcin[1], P. Siedlecki[2], J. Gawor[3], R. Gromadka[3], T. Jagielski[1]
[1]University of Warsaw - Faculty of Biology, Department of Applied Microbiology, Warszawa, Poland
[2]University of Warsaw - Faculty of Biology, Department of Systems Biology, Warszawa, Poland
[3]Institute of Biochemistry and Biophysics, DNA Sequencing and Oligonucleotides Synthesis Unit, Warsaw, Poland

Backgrounds

The genus *Prototheca* comprises unicellular, achlorophyllous, yeast-like algae widely distributed in the environment. Four out of seven currently postulated species are pathogenic to humans and animals, being the causative agents of protothecosis.

State-of-the-art methods of *Prototheca* spp. identification and discrimination include microscopic assessment of cell morphology, evaluation of carbohydrate and alcohol assimilation patterns, and three PCR based methods, which focus on analysis of rDNA operon. The molecular identification methods have been designed for identification of *P. blaschkeae*, *P. zopfii* gen. 1, and *P. zopfii* gen. 2 and are not suited for differentiation of other postulated species.

Moreover, our studies have shown (not published) that each *Prototheca* species has multiple divergent copies of rDNA operon in its genome. This poses technical problems for analysis of PCR products and sequencing.

Objectives

The study aims at proposing an accurate, easy-to-use, quick, and cheap method for differentiation of all *Prototheca* species currently known as an alternative approach to current typing methods.

Methods

Based on whole genome sequencing of reference strains of eight *Prototheca* species (genotypes) (not published) eight draft genomes were assembled, for which genes were predicted *in silico*. Cross-examination of the drafts revealed a total of 187 genes that were shared by all eight strains. Of these genes, two single-copy genes were selected based on their discriminative power in phylogenetic analyses. For these genes primer pairs were designed, PCR products amplified and sequenced.

Conclusions

Two new PCR-sequencing assays based on single-copy genes, i.e. *CYTB* and *EF1α*, are proposed. These two phylogenetic markers enable distinction of all seven known species, offering a useful tool in the identification algorithm of *Prototheca* algae.

The study was financed by the National Science Centre grants «PRELUDIUM» (2013/09/N/NZ2/00248) and «SONATA» (2014/15/D/NZ7/01797).

FEMS7-3120
New Methods and Techniques

DE NOVO ASSEMBLY OF MICROBIAL GENOMES FROM HUMAN GUT METAGENOMES USING BARCODED SHORT READ SEQUENCES

A. Bishara[1], E. Tkachenko[2], E. Moss[2], T. Andermann[2], J. Kang[2], S. Batzoglou[1], A. Bhatt[2]
[1]Stanford University, Computer Science, Stanford, USA
[2]Stanford University, Genetics, Stanford, USA

Backgrounds

Assembling genomes and defining strain-level architecture within complex microbial communities are challenging with current shotgun sequencing approaches. Existing methodologies do not capture structural differences between closely related co-occurring strains such as those arising from horizontal gene transfer and insertion sequence mobilization. Recent techniques partition large DNA molecules, then barcode short fragments derived from them, to produce short-read sequences containing long-range information. These techniques combine high nucleotide accuracy and low input mass requirements with the capacity to resolve repeated sequences within metagenomes.

Objectives

We present a novel application of short-read barcoding techniques to metagenomic samples, and Athena, an assembler that uses these barcodes to produce improved metagenomic assemblies.

Methods

We compare our approach utilizing the 10x Genomics Gemcode platform against conventional shotgun sequencing techniques in a mock bacterial mixture and a clinical gut microbiome time series. Athena assemblies are validated with Sanger sequencing. In addition, we perform RNA sequencing to investigate transcriptional effects of newly assembled sequences.

Conclusions

In the mock bacterial mixture, we successfully assemble and genomically place multiple copies of the highly conserved 16S/23S ribosomal RNA operon, which is present in a single disconnected copy in the conventional assembly. In the clinical time series, we significantly improve draft completeness, uncover strains of Bacteroides caccae differing in the positions of transposon integration, and find the abundance of these strains to fluctuate widely over the course of treatment. RNA sequencing reveals overexpression of antibiotic resistance genes coinciding with both antibiotic administration and the appearance of proximal transposons harboring a putative bacterial promoter.

FEMS7-1812
New Methods and Techniques

MONITORING THE RESPONSE OF STAPHYLOCOCCUS AUREUS TO ANTIMICROBIAL PLANT PHENOLICS BY MULTIPARAMETER FLOW CYTOMETRY

L. Bouarab Chibane[1], *P. Degraeve*[1], *N. Oulahal*[1]
[1]*Université Claude Bernard Lyon1, Genie Biologique, Bourg en Bresse, France*

Backgrounds

Screening of antibacterial biomolecules is still primarily assessed at the population or colony level. However, it is accepted that a bacterial cells population exposed to a stress can cause the appearance of different cell populations including sub lethally compromised cells which could be defined as viable but non culturable (VBNC). Recent advances in flow cytometry namely in multi parameter flow cytometry (MP-FCM) provide the opportunity to obtain high-speed information at real time on damage at single-cell level.

Objectives

The aim of this study was to examine the possibility to corroborate MP-FCM analysis with additional techniques (culturable cells enumeration, effect on liposomes as biophysical membrane models) to elucidate the mechanism of action of plant phenolics active against *Staphylococcus aureus*.

Methods

Fluorescent probes such as propidium iodide, Syto9®, Bis (1,3-dibutylbarbituric acid) and 5(6)-Carboxyfluorescein diacetate were analyzed by flow cytometry to examine vital functions like the membrane integrity, the membrane potential and metabolic activity of *S. aureus* cells exposed to various antimicrobial plant phenolics (5,7-dihydroxy-4-phenylcoumarin, isobutyl-4-hydroxybenzoate, epigallocatechin gallate and 5,8-dihydroxy-1,4-naphtoquinone).

Conclusions

Comparison of MP-FCM analyses with culturable cells enumeration and observation of the effect on liposomes integrity of various plant phenolics revealed that while compounds such as 5,7-dihydroxy-4-phenylcoumarin and isobutyl-4-hydroxybenzoate were bactericidal, they neither affected the integrity of the membrane of *S. aureus* cells, nor the integrity of liposomes.

FEMS7-3005
New Methods and Techniques

IMPLEMENTATION OF AN AUTOLYSIS SYSTEM AS A TOOL FOR FUNCTIONAL METAGENOMICS ANALYSIS

J. Cárcel Márquez[1], E.M. Camacho Fernández[1], A. Flores Díaz[1], E. Santero Saturino[1]
[1]Centro Andaluz de Biología del Desarrollo/CSIC/Universidad Pablo de Olavide/Junta de Andalucía, Departamento de Biología Molecular e Ingeniería Bioquímica, Sevilla, Spain

Backgrounds

Functional metagenomic allows for the identification of novel activities of interest present in uncultured microorganisms. Unfortunately, this strategy is limited, among other factors, by the accessibility of the heterologous enzyme to the substrate. For intracellular activities, the substrate must enter the bacterial cell to allow detection, while for extracellular activities the host must be able to secrete the enzyme to the growth medium. This way the efficiency of the screening could be limited.

Objectives

The main purpose of this work is to develop an autolysis system that, in response to an inducer, lysates bacteria releasing their content into the growth medium. This system must also permit survival of a proportion of the bacterial population to allow the recovery of positive clones

Methods

We have implemented an inducible autolysis system previously developed in our laboratory. It is based in the lysis operon of lambda phage and responds to anhydrotetracycline as an inducer. We have demonstrated that the autolysis system allows detection of intracellular activities and the recovery of positive clones. Subsequently we carried out a screening for cellullase activity in the presence or absence of the autolysis system to compare the efficiency of cellulose activity detection

Conclusions

We have demonstrated that the autolysis system is necessary for the detection of intracellular activities. Additionally, the system improves detection of positive clones for cellullase activity. In all cases, the lysis system allows recovery and subsequent confirmation of positive clones. In summary, our system is an effective tool to improve functional metagenomic analysis.

FEMS7-1795
New Methods and Techniques

WHOLE GENOME SEQUENCE OF NOCARDIA CERRADOENSIS STRAIN CNM 20130759, ISOLATED FROM HUMAN IN A PRIMARY CUTANEOUS INFECTION

G. Carrasco[1], S. Monzón[2], N. Garrido[1], P. Jiménez[3], I. Cuesta[2], M.J. Medina-Pascual[1], J.A. Sáez-Nieto[1], S. Valdezate[1]
[1]National Centre of Microbiology / Institute of Health Carlos III, Taxonomic Reference and Research Laboratory, Majadahonda Madrid, Spain
[2]National Centre of Microbiology / Institute of Health Carlos III, Bioinformatics Department, Majadahonda Madrid, Spain
[3]National Centre of Microbiology / Institute of Health Carlos III, Genomic Department, Majadahonda Madrid, Spain

Backgrounds

Nocardia cerradoensis is an unusual member of the *Nocardia nova* complex and the only available draft genome belongs to an environmental strain.

Objectives

First whole genome sequencing (WGS) of a clinical *N. cerradoensis* strain, isolated from an immunosuppressed 52-year-old woman with internal infection after infiltration.

Methods

The *de novo* draft genome sequencing was performed on the Illumina® NextSeq 500 sequencing system using the fragment library generated by the Nextera. After correction (fastQC v0.11.3) and removing adapter (Trimmomatric v0.36), sequence was processed and assembled using SPAdes (vs3.8.0). Later, the QUAST quality control was applied. 16S rRNA, *gyrB*, MLSA pattern (16S-*gyrB*-*hsp65*-*secA1*) were determined by PCR.

Conclusions

N. cerradoensis strains CNM20130759 showed a genome size of 8,9 Mb, including 972 contigs (120 contigs ≥ 1000 bp; minimum, 222 bp; maximum, 1035523bp; N50, 394,043bp) and a GC-content of 67.74%. No CRISPR repeats were founded. The housekeeping and intrinsic genes (*gyrB, rpoA, rpoB, secA, hsp65*, *folP, trpB, cobQ, soda, sodC, inhA, nrdF, nrdB, nrdE, nrdH, …)*, resistance and virulence genes (ATP-binding cassette, *ddpA*, fibronectin-binding protein, sortase A, ESC-1, serine protase, *tlyC*, *katA, katE* and *katG, murA, Int-Tn, stp, tetR,* NCRCNM_01157, NCRCNM_01690*, bmrR, yfmO, mdtG, ermY, qacA, drrA, surG, mdtL, bcr, marR, mmr,*…) were identified. The characteristics genes of nearby genus as *Mycobacterium* (Insertion elements IS6110 and IS5376, universal stress protein Rv2005c, KsdD-like Rv0785, sensor TcrY, multidrug efflux Rv0194, Rv1459c …) were also found. MLST is a good approach for identification, but WGS became the definitive tool for the identification and the understanding of the virulence of *N. cerradoensis.*

FEMS7-0864
New Methods and Techniques

COLD ATMOSPHERIC PLASMA AS A DECONTAMINANT OF MYCOPLASMA-INFECTED CELLS.

Y. Chalenko[1], E. Sysolyatina[1], I. Rakovskaya[2], M. Vasiliev[3], O. Petrov[4], V. Fortov[5], S. Ermolaeva[1]
[1]Gamaleya Research Center of Epidemiology and Microbiology, laboratory of ecology pathogens, Moscow, Russia
[2]Gamaleya Research Center of Epidemiology and Microbiology, laboratory of mycoplasma, Moscow, Russia
[3]Joined Institute of High Temperatures, laboratory of dusty plasma, Moscow, Russia
[4]Joined Institute of High Temperatures, laboratory of low-temperature plasma, Moscow, Russia
[5]Joined Institute of High Temperatures, High Temperatures RAS, Moscow, Russia

Backgrounds

Mycoplasma spp. are the smallest bacteria lacking the cell wall [1]. Many species represent a serious problem for industrial biotechnology being common contaminants of cell lines [1]. Cold atmospheric plasma (CAP) is a flow of partly ionized gas that has an ambient temperature. CAP has unspecific bactericidal activity due to charged particles, active oxygen and nitrogen species and UV [2].

Objectives

To establish a bactericidal effect of cold atmospheric plasma on *Mycoplasma hominis.*

Methods

To investigate a direct effect, *M. hominis* plated on agar or HEp-2 cells infected with 10^7 CFU ml^{-1}*M. hominis* were treated with CAP, described previously [2], for 2 or 5 min. Survivors were counted in 72 h.For an indirect plasma effect investigation the cultural medium DMEM was treated with plasma for 5 min and then added to infected cells. *M. hominis* survivors were plated on agar and counted in 72 h.

Conclusions

M. hominis showed an outstanding CAP resistance in comparison with other bacteria [2,3]. 5-minutes direct plasma treatment of infected cells reduced the amount of mycoplasmas twice, but had a toxic effect on HEp-2 cells. Indirect treatment was not toxic for cells and caused 10 fold drop of bacteria load. Thus, CAP treatment of cultural medium might be useful for decontamination of cell lines infected with *Mycoplasma* spp.

References

[1] S. Razin, R. Germann (eds). Molecular Biology and Pathogenicity of Mycoplasmas, (2002).

[2] S. Ermolaeva et al. *J Med Microbiol.,* **60(1)**, 75-83 (2011)

[3] S. Ermolaeva et al. *J. of Applied Microbiology*, **116.5**, 1129-1136 (2014)

FEMS7-0988
New Methods and Techniques

USE OF ITS1 REGION AS BARCODE MARKER TO DETECT AND CLASSIFY FUNGI IN HUMAN SAMPLES BY ILLUMINA SEQUENCING PLATFORM

P. Corell Escuin[1], D. Pérez-Villarroya[1], M.L. Ferrús Abad[1], A. Artacho Pérez[2], A. Mira Obrador[2], A. Alastruey Izquierdo[3], G. D'Auria[1,4], L. Martínez Priego[1]
[1]FISABIO Public Health, Sequencing and Bioinformatics Service, Valencia, Spain
[2]FISABIO Public Health, Department of Genomics and Health, Valencia, Spain
[3]Instituto de Salud Carlos III, Centro Nacional de Microbiología, Madrid, Spain
[4]CIBER, Epidemiology and Public Health, Madrid, Spain

Backgrounds

The identification of fungal presence in human samples have traditionally relied on disease symptoms, laboratory identification and biochemical tests. Although these methods are still fundamental, there is an increasing move towards molecular diagnosis. Since the spread of Next Generation Sequencing (NGS) technologies, researchers have been on the hunt for reliable DNA barcode markers capable to describe Prokaryota and Eukaryota taxonomy. Specifically, for fungal samples, the Internal Transcribed Spacer (ITS) between 18S and 28S ribosomal genes has been the best candidate barcode marker so far, but still there is no consensus about the most suitable pair of primers targeting this region.

Objectives

The efficiency and specificity of a renowned pair of primers targeting the ITS1 region have here determined and an optimization protocol is proposed for Illumina MiSeq sequencing platform.

Methods

A mock community of 6 different fungal species causing human diseases has here tested checking for the influence of starting DNA amount and their relative dominance. Besides, the primers specificity was tested.

Conclusions

The use of tested primers, although statistical analysis of sequences distribution revealed some biases due to PCR and library preparation, faithfully reflects the initial proportion of the species. Besides, we observed no unspecific amplification of control human DNA. Finally, the results confirm the reliability of tested primers targeting ITS1 for fungi detection and characterization by PCR reaction, library preparation and Illumina based sequencing for clinical molecular diagnosis.

FEMS7-0921
New Methods and Techniques

BIOGENIC SELENIUM NANOPARTICLES: A NOVEL STRATEGY TO FACE ANTIBIOTIC RESISTANCE?

E. Cremonini[1], *M.M. Lleo*[1]
[1]*University of Verona, Diagnostic and Public Health, Verona, Italy*

Backgrounds

Tailored metal nanoparticles with desired physico-chemical properties have been proposed as a new line in the battle against antibiotic-resistant microorganisms.

Objectives

In this study, biogenic Selenium Nanoparticles (SeNPs) have been tested as an alternative therapy for antibiotic resistant bacteria.

Methods

The strain *Stenotrophomonas maltophilia* SeITE02 was used to produce biogenic NPs. The antibacterial activity of SeNPs was tested against several clinical strains from *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Staphylococcus haemolyticus* and *Staphylococcus aureus*. MICs of SeNPs and antibiotics were determined according to the CLSI broth microdilution method. The NPs inhibitory and disruptive biofilm activity was determined by Crystal Violet staining and conventional plating. Bioiflm biomass was determined also by observation at fluorescence microscope.

Conclusions

SeNPs demonstrated antibacterial activity with low MIC values (8-16 µg/ml) against some of the tested strains and proved capable of inhibiting the synthesis of biofilm and disaggregating the polysaccharide matrix at concentrations ranging between 50 and 100 µg/ml. The quantification of culturable cells points out the antibiofilm potential of the SeNPs causing a 2-8 $\log_{10}$ CFU/ml reduction. Experiments to test the possible synergistic antibiofilm activity of SeNPs and antibiotics are still ongoing.
Biogenic SeNPs showed a good antibiofilm and antibacterial activity with low MICs value for a number of clinical isolates. SeNPs can be considered biocompatible structures that could be administered, either alone or in combination with antibiotics, in new therapeutic strategies to inhibit the growth of resistant pathogens or to facilitate the penetration of microbial biofilms.

FEMS7-0611
New Methods and Techniques

A NOVEL NUCLEIC-ACID BASED BIOSENSOR FOR VIBRIO SPP. DETECTION IN AQUATIC ENVIRONMENTS

E. Da-silva[1], J. Baudart[2], L. Barthelmebs[1]
[1]Université de Perpignan Via Domitia, Laboratoire Biocapteurs Analyses Environnement, Perpignan, France
[2]Sorbonne Universités- UPMC Univ. Paris 06- USR 3579, LBBM- Observatoire Océanologique, Banyuls-sur-Mer, France

Backgrounds

The water resource is regularly monitored for its bacteriological quality through the detection of fecal indicator bacteria, such as *Escherichia coli*, using culture-based methods. Nevertheless, non fecal pathogens are widely distributed in aquatic environment, and due to environmental stresses, may enter into a viable but non culturable state becoming non detectable by cultivation. Nucleic acid-based biosensors are innovative analytical tools allowing a rapid detection of bacteria and present several advantages. These devices are cost-effective, robust, sensitive, specific, quantitative and do not required genes amplification.

Objectives

In the present study, a novel nucleic acid-based biosensor was developed for the monitoring of *Vibrio* spp., including well-known pathogenic species.

Methods

This biosensor was based on a sandwich hybridization assay in which nucleic acid target (RNA) was bound between an immobilized capture probe and a labeled signal probe. The hybridization was then revealed by an enzymatic system, leading to a signal proportional to the entrapped target.

Conclusions

In a first step, probes were validated using synthetic targets and detection limit was determined as 100 pM. In a second step, specificity was checked by using RNA extracted from a panel of 31 environmental bacterial strains. The biosensor allowed a good discrimination between the *Vibrio* and the non-*Vibrio* strains and detection limit of 5 $ng.\mu L^{-1}$ of total RNA was obtained. Finally, the device was successfully applied to the analysis of spiked and natural environmental samples.
In order to develop miniaturized devices that could be directly implemented on site, transfer towards an electrochemical detection is in progress.

FEMS7-0891
New Methods and Techniques

THE OCCURRENCE AND ASSOCIATION OF FUSARIUM SPECIES AND THEIR EMERGING MYCOTOXINS IN WHEAT, MAIZE AND LEEK

M. Decleer[1], A. Rajkovic[1], K. Audenaert[2], S. De Saeger[3]
[1]Ghent University, Department of Food Safety and Food Quality, Gent, Belgium
[2]Ghent University, Department of Applied biosciences, Gent, Belgium
[3]Ghent University, Department of Bioanalysis, Gent, Belgium

Backgrounds

The emerging *Fusarium* mycotoxins are a class of compounds that are attracting increasing interest due to their presence in high concentrations in many food products and their potential toxicity towards animals and humans.

Objectives

The objectives of this research were (i) to evaluate the natural occurrence of beauvericin (BEA) and enniatins A, A1, B and B1 (ENNs) in wheat and maize collected from trial fields in Flanders, Belgium, (ii) to estimate a possible correlation between fungal colonization by *Fusarium* species and accumulation of BEA and ENNs and (iii) to characterize the production of BEA, ENNs and moniliformin (MON) by *Fusarium* isolates in an interaction with leek.

Methods

704 wheat and maize samples were collected during the harvest of 2015 and 2016 and analysed for BEA and ENNS by LC-MS/MS according to Decleer et al. (2016). Leek plants infected with *F. avenaceum* were analyzed for BEA, ENNs and MON with an UPLC–MS/MS method developed and validated in the framework of this study. To assess the link between *Fusarium* species and their mycotoxin production, Q-PCR was used to measure the presence of *Fusarium* species.

Conclusions

The results indicated that all wheat samples were contaminated with at least one of the emerging mycotoxins with BEA, ENN B and ENN B1 as the most abundant mycotoxins in Belgian maize and wheat samples. Since the incidence and concentrations of the artificially inoculated leek plants were low, we assume that these mycotoxins are most likely not involved in the interaction of *F. avenaceum* with its host leek.

FEMS7-0941
New Methods and Techniques

INFLUENCE OF CEREULIDE ON THE MITOCHONDRIAL FUNCTION OF CACO-2 AND HEPG2 CELLS USING EXTRACELLULAR FLUX ANALYSIS

M. Decleer[1], S. De Saeger[2], A. Rajkovic[1]
[1]Ghent University, Department of Food Safety and Food Quality, Gent, Belgium
[2]Ghent University, Department of Bioanalysis, Gent, Belgium

Backgrounds

Cereulide (CER) is a lipophilic cyclododecadepsipeptide produced by Bacillus cereus. This toxin is known to induce food poisoning, sometimes related with liver failure and even fatal outcome. In contrast with doses associated with food poisoning, recent prevalence data demonstrated relatively low concentrations of cereulide in rice and pasta dishes. The effects of repeated exposure to low levels of cereulide is largely unknown and can lead to(sub)chronic harms.

Objectives

The goal was to provide insight into the impact of a continuous exposure of low doses of CER on metabolic responses of Caco-2 and HepG2 cells as models for intestinal and liver toxicity.

Methods

Caco-2 and HepG2 cells were exposed to food-relevant low concentrations of CER to investigate the effect of a longer exposure. To explore the mechanisms involved in the mitochondrial function, the Seahorse Bioscience XFe24 analyzer (Massachusetts, USA) was used in combination with well-established assays for mitochondrial activity (MTT) and changes in protein content (SRB (sulforhodamine B)). The effects of CER on the mitochondrial oxygen consumption rate (OCR) were assessed using the Seahorse Bioscience XF Cell Mito Stress Test assay kit. High-resolution mass spectrometry was used to unravel the metabolic profile of CER.

Conclusions

The three-day treatment with low concentrations of CER on mitochondrial respiration in Caco-2 cells showed perturbations in mitochondrial respiration at 0.125 ng/ml. These in vitro data suggest that repeated exposure of CER might injure intestinal cells even at relative low doses. Cereulide appear to be more toxic than other cyclodepsipeptide toxins with ionophoretic properties like valinomycin and beauvericin.

FEMS7-2169
New Methods and Techniques

FILMARRAY GI® PANEL FOR DETECTION OF ENTERIC PATHOGENS IN STOOL SAMPLES IN CHILE

F. Mauricio[1], L. Yohanna[1], H. Juan[1], B. Pablo[1], G. Sebastian[1], S. Cynthia[1], F. Camila[1], T. Juan[1]
[1]Laboratorio de Biología Molecular, Clínica las Condes, Santiago, Chile

Backgrounds

Gastrointestinal infections remain a major public health problem worldwide and its diagnosis is one of the main challenges. In the last years, several molecular techniques for the detection of multiple pathogens have been developed, allowing to determine the etiology of infection. In 2014, the FDA released for clinical use the Filmarray GI® panel that allows detection of 23 pathogens (14 bacteria, virus 5 and 4 parasites) within an hour.

Objectives

To show and analyze the experience of Filmarray GI® panel in the Molecular Biology Laboratory of Clinica las Condes, Chile

Methods

A cross-sectional observational study that includes the results of 2127 stool samples tested by Filmarray GI® panel from January 2015 to January 2016.

Conclusions

Of the 2127 tests requested around one third were negative and positive samples with one to eight pathogens were detected. High prevalence of diarrheogenic *E. coli* and co-infections was observed. Implementation of a 24/7 molecular testing significantly decreased the average turn-around-time (TAT) of this panel from hours to 2.5 hours. This work presents the experience of using FilmArray GI® panel as a tool for diagnosis of gastrointestinal infections, which highlights the large number of positive samples for a microorganism and co-detection of enteric pathogens. The 24/7 service provided detection results to clinicians in a timely manner.

FEMS7-2387
New Methods and Techniques

CONSTRUCTION OF A PROMOTER STRENGHT SCREENING VECTOR IN BACILLUS SUBTILIS BASED ON A GENE ENCODING A SUPERFOLDER GREEN FLUORESCENCE PROTEIN COMBINED WITH FACS

C. Gago Córdoba[1], *W. Meijer*[1]
[1]*Centro de Biología Molecular Severo Ochoa, Virología y Microbiologia, MADRID, Spain*

Backgrounds

Identification of promoters and determination of their strength and regulation is crucial for understanding the role of downstream-located genes. RNAseq or qPCR can identify and monitor promoter strength. However, these methods are time consuming, expensive and do not allow easy testing of the promoters in different settings. Systems based on reporter genes are generally used for these studies. Systems are based on lacZ fusions are laborious and provide only the mean expression profile of all cells within the population. GFP is an excellent reporter allowing rapid, reproducible analysis at single cell level. However, convenient systems for Bacillus subtilis are lacking, principally because most versions of the gfp protein variants do function well at 37 degrees.

Objectives

Developing a convenient promoter characterization system for B. subtilis using as reporter a superfolder GFP-encoding gene that is optimized for stable expression in B. subtilis.

Methods

PCR-based cloning was performed to construct a B. subtilis integration vector containing a special gfp reporter gene apt for promoter study analysis.

Conclusions

We have developed a B. subtilis integration vector containing a promoterless special gfp gene that has been improved in several ways for optimal use in B. subtilis and containing three unique restriction sites upstream of the RBS.

Variants of the system were constructed in which inducible promoters with different strengths were cloned in front of the reporter gene and these were used to prepare calibration profile to determine the relative strength of promoters of interest by FACS.

FEMS7-2162
New Methods and Techniques

ASPERGILLUS FUMIGATUS ERG4B (AFU1G07140): A USEFUL TOOL FOR TYPING PURPOSES

R. Garcia-Rubio[1], *G. Santoni*[2], *M.C. Monteiro*[3], *G. Riaran-Moreno*[3], *L. Alcazar-Fuoli*[3], *E. Mellado*[3]
[1]*Spanish National Center for Microbiology, Mycology, Majadahonda Madrid, Spain*
[2]*Hospital Británico de Buenos Aires, Infectious Diseases Department, Buenos Aires, Argentina*
[3]*National Center for Microbiology, Mycology, Majadahonda Madrid, Spain*

Backgrounds

Aspergillus fumigatus is a saprotrophic mold responsible for invasive aspergillosis in immunocompromised hosts. Ergosterol is the main sterol of fungal membranes and the target of some antifungal agents. In *A. fumigatus* ergosterol pathway, some enzymes have two homologs such as the sterol C-24 reductase, encoded by *erg4A* and *erg4B*, which catalyze a final step of the ergosterol biosynthesis. Deletion of *erg4* in *Saccharomyces cerevisiae* and *Fusarium graminearum* changes their azole susceptibility profile.

Objectives

1.- To improve a genotyping method previously described (Garcia-Rubio et al., 2016) based on hypervariable tandem repeats within exons of surface protein coding genes (TRESP) by adding *A. fumigatus erg4B* sequencing information.

2.- To investigate whether differences in *erg4B* tandem repeats are related to the azole susceptibility profile of *A. fumigatus* strains.

Methods

One hundred and fifty *A. fumigatus* strains with clinical origin (105 azole susceptible and 45 azole resistant) were used in this study. They were previously characterized by PCR amplification and sequencing of three genes: Afu3g08990 (CSP), Afu2g05150 (MP2), and Afu6g14090 (CFEM). Afu1g07140 (*erg4B*) sequences were added for improving typing discrimination.

Conclusions

The TRESP typing discrimination power was improved when *erg4B* sequences were added as a typing target. So far no relation between the size of tandem repeat integrations and azole susceptibility profile was obtained. Further work with *erg4B* mutants will confirm its role in the ergosterol biosynthesis and its relation with azole susceptibility.

FEMS7-3043
New Methods and Techniques

DISCRIMINATION AMONG SALMONELLA ENTERICA SEROTYPES BY MULTI-LOCUS SEQUENCE TYPING (MLST) SCHEME AS A GLOBAL PROSPECTIVE STRATEGY

R. Ghaderi[1], K. Tadayon[1], P. Khaki[2], S. Moradi Bidhendi[2], N. Mosavari[3], R. Banihashemei[1], M. Sekhavati[1], A. zaree[2], G. Mehdi[2]
[1]Razi Vaccine and Serum Research Institute- Agricultural Research- Education and Extension Organi zation AREEO, Aerobic Bacterial Vaccine, KARAJ, Iran
[2]Razi Vaccine and Serum Research Institute- Agricultural Research- Education and Extension Organi zation AREEO, Microbiology, KARAJ, Iran
[3]Razi Vaccine and Serum Research Institute- Agricultural Research- Education and Extension Organi zation AREEO, Tuberculin, KARAJ, Iran

Backgrounds

Salmonella as a major food-borne disease has imposed significant public health and economic loss along the globe. Traditionally, serotyping has lots of shortcomings, on the other hand, results acquired by most of molecular typing methods aren't reproducible. Also these techniques should be able to determine the origin of a single globally distributed clonal lineage.

Objectives

To solve all mentioned problems related to conventional and molecular typing method, MLST scheme has been implemented as a generic, repeatable, robust and portable technique.

Methods

Having a previous history of clonal population structure among 76 *Salmonella* Enteritidis by using MLST technique, this study was designed to evaluate the discrimination ability of this method among different *Salmonalla* serotypes. 4 DNA templates belonging to 3 different serogroup D1, B and C1and One standard *salmonella* Enteritidis were exploited in this study. Three out of seven MLST house-keeping genes including hisD, thrA, sucA were considered to be amplified.

Conclusions

While we observed a clonal population group among our *Salmonella* Enteritidis isolates, we successfully differentiated 3 most frequent Iranian serotype including Enteritidis, Typhimurium and Infantis, in different clustal groups.
Generally, molecular typing methods are intended to tackle two different levels of epidemiological problems. In one hand localized outbreak of disease in a short period of time and on the other, relation between strains causing a disease in different geographic areas with a longer period. This study proves MLST method is a satisfactory platform to provide all these needs and can be regarded as a desirable prospective global typing strategy.

FEMS7-3045
New Methods and Techniques

DEVELOPING MLVA TECHNIQUE AS A COMPROMISED APPROACH TOWARD MOLECULAR CHARACTERIZATION OF PASTEURELLA MULTOCIDA

R. Ghaderi[1], K. Tadayon[1], A. Jabbari[1], A. sanchooli[1], A. Arab[1], R. Banihashemi[1], M. SEKHAVATI[1]
[1]Razi Vaccine and Serum Research Institute- Agricultural Research- Education and Extension Organi zation AREEO, Aerobic Bacterial Vaccine, KARAJ, Iran

Backgrounds

Multi locus variable number tandem repeat analysis as a PCR-based technique scrutinizes short tandem repeats within amplified DNA. These tandem repeat units show frequent variation in the number of copies by slippage strand misalignment throughout DNA synthesis. Different numbers of tandem repeat units makes different fragment lengths in a way that each discrete sized fragment called as unique "allele" for the locus under study. Razi Vaccine and Serum Institute has a long history of production of vaccine against fowl cholera and Haemorrhagic septicemia using indigenous strains.

Objectives

In order to genetically characterize these vaccine strains, it was tried to set-up this technique over *Pasteurella multocida* while there had been no evidence of any similar attempts so far.

Methods

Employing Tandem Repeat Finder software, *Pasteurella multocida* strain PM70 as the main complete genome was gone under TRs investigation process. More over 9 other complete genome strains including: ATCC43137, HB01, OH4807, PMTB2.1, 3480, HB03, HN06, NC-006300-fna, and NC-016808-fna were compared by selected tandem repeats.

Conclusions

Three microsatellites markers with 6, 9 and 12 bp size were selected entitled VNTR6,VNTR9and VNTR12. Using Primer 3 software under NCBI website 3 pairs of primers were designed to incorporate Amplicons less than 800 bps in length encompassing the Tandem repeat region in the middle. Optimization process was done to harmonize all 3 amplification protocols. 2 vaccine strains and 4 field isolates were successfully gone under MLVA investigations. Our findings proved the feasibility of this technique that can be employed as an appropriate way to characterize *Pasteurella multocida* isolates.

FEMS7-1970
New Methods and Techniques

NOVEL ISOLATION AND CULTURE APPROACHES TO IMPROVE THE RECOVERY OF LICHEN ASSOCIATED BACTERIA USING LICHENIC EXTRACTS

E. G Biosca[1], R. Flores[1,2,3], R. D Santander[1], J.L. Díez-Gil[4], E. Barreno[2,3]
[1]Universitat de Valencia, Microbiología y Ecología, Burjassot, Spain
[2]Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Botánica, Burjassot, Spain
[3]Universitat de Valencia, Botánica, Burjassot, Spain
[4]Instituto de Investigación Sanitaria La Fe, Grupo RETRACAR, Valencia, Spain

Backgrounds

Lichens, commonly considered mutualisms between a mycobiont and one or more photobionts, are now recognized as multispecies symbiosis in which non-photosynthetic bacterial communities are stable, specific and structurally integrated partners. The diversity and functional roles of these symbiotic bacteria within the lichen have recently begun to be studied, but mainly by culture-independent approaches. This is because of difficulties in their isolation and culture on standard media that do not mimic lichen nutrients. Nevertheless, to culture as yet unculturable bacterial symbionts is essential to clarify their contribution to the lichen symbiosis, but also to explore their biotechnological potential and the description of new taxa.

Objectives

The objective was to improve the recovery of lichen associated bacteria by developing innovative isolation and culture approaches.

Methods

We evaluated the effect of thalli washing time, and different disinfection treatments and processing protocols, as well as newly developed growth media enriched with novel lichen extracts. Once optimized the methodology, it was applied for the isolation of bacteria from the lichens *Pseudevernia furfuracea*, *Ramalina farinacea* and *Parmotrema pseudotinctorum*.

Conclusions

The developed methodology allowed significant increases in the number and diversity of lichen associated culturable bacteria, which makes lichens an important source of new microorganisms. Further, it allowed the growth of previously uncultured lichen symbiotic bacteria. This methodology, which is patent granted (ES2575752, 2017), is also applicable to other microorganisms of these lichen species and others. (GV-PROMETEOII/2013/021 and UV-INV-AE 112-66196).

Reference: Biosca EG et al. 2016. PLoS One. doi: 10.1371/journal.pone.0160328.

FEMS7-1059
New Methods and Techniques

CIRCULAR DICHROISM SPECTROSCOPY REVEALS NEW POSSIBILITIES IN DETERMINATION OF STERIGMATOCYSTIN IN AQUEOUS SOLUTIONS

D. Jaksic Despot[1], M. Šegvić Klarić[2], I. Piantanida[3]
[1]Faculty of pharmacy and biochemistry, Department of microbiology, Zagreb, Croatia
[2]University of Zagreb- Faculty of pharmacy and biochemistry, Department of microbiology, Zagreb, Croatia
[3]Ruđer Bošković Institute,
Division of organic chemistry and biochemistry- Laboratory for biomolecular interactions and spectros copy, Zagreb, Croatia

Backgrounds

Sterigmatocystin (STC) is a mycotoxin produced by many fungal species, ubiquitous in working and living environment. STC exhibits hepatotoxic, nephrotoxic, mutagenic and carcinogenic effects representing a significant risk to human and animal health. LC-MS is the most commonly used technique for the STC detection. Possible complications like matrix effects on ionisation efficiency make use of isotopically labelled STC internal standard inevitable, in addition to laborious sample preparation. Circular dichroism (CD) spectroscopy is extensively used to study structural, kinetic and thermodynamic features of chiral molecules. Since STC is chiral molecule CD spectroscopy for its determination in various substrates is worth of exploring.

Objectives

The purpose of this study was to demonstrate CD spectroscopy application in the determination of STC in various aqueous matrices.

Methods

CD spectra of STC aqueous solutions were recorded by standard Jasco J810 setup in 1 cm quartz cuvette at various physicochemical conditions (temperature, organic solvents and salts addition).

Conclusions

STC forms aggregate in aqueous solutions, yielding strong CD signal in 300-400 nm range specific to STC, with the intensity up to 1000:1 compared to the baseline. The CD signal intensity is proportional to STC concentration within 10^{-7} M to 10^{-5} M range, and none of relevant species from food or environment (including structurally similar aflatoxin B1) does not interfere with this CD-signal. These facts strongly encourage an innovative approach to STC monitoring, which could result in a completely new analytical method for the specific determination of STC, as well as studying non-covalent interactions of STC with biomacromolecules.

FEMS7-3254
New Methods and Techniques

GEXPLORE – A NOVEL GENOME-WIDE APPROACH FOR STUDYING HOST-PATHOGEN INTERACTIONS

S. Kambarev[1], V. Kalichuk[1], G. Béhar[1], B. Mouratou[1], L. Marsollier[2], S. Corvec[3], F. Pecorari[1]
[1]CRCINA- INSERM- CNRS- Université d'Angers- Université de Nantes- Nantes- France, Nuclear Oncology Research, Nantes, France
[2]CRCINA- INSERM- Université de Nantes- Université d'Angers- Angers- France, Avenir ATOMycA, Angers, France
[3]CRCINA- INSERM- CNRS- Université d'Angers- Université de Nantes- Nantes- France, Clinical and translational research in skin cancer, Nantes, France

Backgrounds

Display technologies such as Shotgun phage display and ANTIGENome have proven their potential for identification of bacterial adhesins and novel vaccine candidates. However, the coverage of their input genomic libraries is limited by the low-efficient bacterial transformation step.

Objectives

The aim of this work was the development of a completely *in vitro*, ribosome display-based approach for genome-wide identification of host-pathogen interactions without the need for transformation step.

Methods

Genomes of *Staphylococcus aureus*, *Streptococcus gallolyticus* and *Mycobacterium ulcerans* were used. DNA fragmentation (100-1000 bp) was performed with ultrasound. Whole-genome expression libraries were prepared using an alternative GC-based cloning strategy and PCR. Ribosome display selection was performed for 3-4 alternate rounds on plates/beads. Whole genomes, input genomic libraries and selection outputs were sequenced on Illumina MiSeq. In order to validate GeXplore, random genomic libraries of *S. aureus* were selected against human Fc fragment. Subsequently, libraries of *S. gallolyticus* and *M. ulcerans* were selected against patient-derived IgG/IgA preparations.

Conclusions

Completely *in vitro* preparation of genomic expression libraries was achieved with our alternative cloning strategy. Next-generation sequencing revealed high genomic library coverage which, interestingly, was directly proportional to the G+C content of the input genome. After three selection rounds the output consisted mainly of fragments, encoding the IgG-binding domains of the staphylococcal proteins Sbi and SpA. Thus, GeXplore showed high specificity on a genome-wide scale. Finally, we used our method to identify potentially immune-relevant proteins of *S. gallolyticus* and *M. ulcerans*. In conclusion, GeXplore has the potential to streamline the host-pathogen interactome analysis.

FEMS7-1525
New Methods and Techniques

RAPID AND SIMPLE DETECTION OF LIPOPOLYSACCHARIDE IN DRINKING WATER USING SURFACE IMMOBILIZED POLYMYXIN B

H.J. Kang[1]
[1]*Korea University, School of Civil- Environmental and Architectural Engineering, Seoul, Republic of Korea*

Backgrounds

Lipopolysaccharide (LPS) is the main component of the cell walls of gram-negative bacteria and a potent initiator of inflammatory reactions in mammalians. It is necessary to monitor LPS in drinking water, because the lysis of gram-negative bacteria and LPS dispersion may result in various complications such as diarrhea, bleeding, fever, etc. These days the Limulus amoebocyte lysate (LAL) assay is the most popular, but the assay is not suitable to monitor LAL in drinking water containing high concentration of divalent cations. It is thus needed a new assay to be insensitive to the presence of divalent cations.

Objectives

Polymyxin B is produced by *Bacillus polymyxa* and attenuates gram-negative infections by binding to LPS. The binding is reported not to be strongly affected by divalent cations. The aim of this study was to develop a new method using polymyxin B to overcome the disadvantages of the LAL assay.

Methods

Polymyxin B is initially immobilized onto the surface of a 96-well plate using a silane coupling agent. Then, a water sample containing LPS and known amount of FITC-LPS were simultaneously added to the polymyxin B immobilized 96-well plate for competitive binding. Fluorescence intensity was analyzed using a multimode plate reader for assaying LPS in the sample.

Conclusions

The newly developed method was less sensitive to pH, temperature, and divalent cations than the LAL assay. Additionally, the method could detect LPS down to 0.07 EU/mL within 15 minutes. These results suggest that the method is efficient in monitoring LPS in drinking water.

FEMS7-3225
New Methods and Techniques

INTRODUCTION OF CULTURE INDEPENDENT METHODS IN THE ROUTINE MONITORING OF MICROBIAL QUALITY OF DRINKING WATER

A. Knezev[1], M. Taucer-Kapteijn[1], E. Penders[1], S. Strating[1], D. Kloes[2], C. de Vries[2], R. Broks[3], R. Steen[1]
[1]Het Waterlaboratorium, Advice, Haarlem, The Netherlands
[2]Het Waterlaboratorium, Biology, Haarlem, The Netherlands
[3]Het Waterlaboratorium, Management, Haarlem, The Netherlands

Backgrounds

The majority of standard methods for microbiological control of drinking water is based on cultivation. Recent developments in microbiology provided a variety of molecular alternatives for the assessment of drinking water quality. Widespread use in research demonstrated the clear benefits of molecular methods. However, lack of a framework for the comparison of data gained by molecular, cultivation independent, techniques with data gained by standard, cultivation-based, techniques represents an important obstacle in the implementation of molecular methods in routine monitoring.

Objectives

Develop a framework for the comparison of molecular methods to cultivation based standards.

Methods

Alternatives for general (GMQ) and hygienic (HMQ) microbial water quality monitoring are discussed. For the GMQ flow-cytometry (FCM) and adenosine three-phosphate (ATP) concentration are compared to heterotrophic plate count (HPC). For HMQ, RNA based detection (RT-PCR) and MALDI-TOF based typing are compared to standard methods for *E.coli* and Enterococci.

Conclusions

Novel methods proved to be faster, highly sensitive and more specific than cultivation based standards. As a result, their application in practice improves operational efficiency and risk control. In spite of higher resolution, new methods also generate uncertainties related to limited information about physiological properties of newly described bacterial species and ecological fate of target components that are used in molecular methods. Wide application of new methods in routine monitoring is crucial to gain real life information, but risks related to uncertainties must be acknowledged and addressed in future scientific research.

FEMS7-2130
New Methods and Techniques

TARGETED METAGENOMICS FOR ANALYSIS OF RESISTOMES (RESCAP1.0)

V. Lanza[1], F. Baquero[1], M. Jose Luis[2], R. Ramos-Ruiz[3], B. Gonzalez-Zorn[4], A. Andremont[5], A. Sanchez-Valenzuela[1], D. Ehrlich[6], S. Kennedy[6], E. Ruppe[6], W. van Schaik[7], R.J. Willems[7], F. de la Cruz[8], T.M. Coque[1]
[1]Hospital Universitario Ramón y Cajal University Hospital- Ramón y Cajal Health R, Servicio de Microbiología, Madrid, Spain
[2]CSIC, Centro Nacional de Biotecnologia, Madrid, Spain
[3]Parque Cientifico de Madrid, Unidad de Genómica, Madrid, Spain
[4]Universidad Complutense de Madrid, Facultad de Veterinaria, Madrid, Spain
[5]Hôpital Bichat, Bactériologie Laboratory, Paris, France
[6]Université Paris-Saclay, MGP MetaGénoPolis- INRA, Paris, France
[7]University Medical Center Utrecht, Medical Microbiology, Utrech, The Netherlands
[8]Universidad de Cantabria y IBBTEC, Biologia Molecular, Santander, Spain

Backgrounds

Antimicrobial resistance is considered a major Global Health challenge. The ensemble of antibiotic resistance genes in particular metagenomes constitutes a “resistome”. Methodological limitations of available high-throughput metagenomic technologies could have seriously influenced our perception about the size and diversity of different resistomes.

Objectives

To develop a targeted capture platform (TCP) for improving the quantitative and qualitative analysis of resistomes (ensemble of genes encoding antibiotic resistance and their precursors in metagenomes). To evaluate the pool of antimicrobial resistance genes (heavy metals, biocides) and plasmid markers (relaxases), relevant for the selection and transmissibility of antimicrobial resistance genes.

Methods

ResCap (a TCP based on NimbleGene-Roche technology), includes probes for 8,667 canonical genes (7,963 to antibiotics, 704 to metal & biocides), 76.000 genes homologous to the former ones (47,806 for antibiotics and 30,794 for biocide and metals) and 2,517 relaxase genes (plasmid markers). Comparison of ResCap with metagenomic shotgun sequencing (MSS) was performed using 17 fecal samples (9 humans, 8 swine).

Conclusions

ResCap significantly improves MSS to detect “gene abundance” (from 2.0% to 83.2%) and “gene diversity” (26 versus 14.9 genes per sample per million of reads, 300 fold by using ResCap), which were calculated using novel bioinformatic tools. New approaches are also provided to compare disparate resistomes. ResCap, the first TCP to analyse resistomes, clearly enhance the sensitivity and specificity of available metagenomic methods, provides the possibility to analyse other genes related to antimicrobial resistance and opens the possibility to accurately study other complex microbial systems.

FEMS7-0176
New Methods and Techniques

DEVELOPMENT OF RECOMBINASE POLYMERASE AMPLIFICATION ASSAYS FOR DETECTION OF COXIELLA BURNETII

Y. Li[1], Q. Yin[2], Y. Qi[1], Y. Shao[1], J. Li[1], Y. Pan[1], S. LI[1], H. Chen[1], T. Xu[3], B. Yang[2], X. Luo[2]
[1]Huadong Research Institute for Medicine and Biotechniques, Medicine and Biotechniques, Nanjing, China
[2]China Pharmaceutical University, School of Pharmacy, Nanjing, China
[3]Nanjing Medical University, School of Basic Medical Sciences, Nanjing, China

Backgrounds

Coxiella burnetii is the causative agents of Q fever.Q fever is highly fatal and listed as a biological warfare agent. Because the clinical symptom of Q fever is not typical, the disease can be easily missed and misdiagnosed. Sensitive, specific and rapid diagnostic tests for the detection of Coxiella burnetii are necessary to accurately and promptly diagnose patients and ensure that they receive proper treatment.

Objectives

To develop recombinase polymerase amplification assays for detection of Coxiella burnetii

Methods

Recombinase polymerase amplification(RPA) assays using a lateral flow test (RPA-nfo) were developed targeting the 23SrRNA gene of Coxiella burnetii. A group of specific primers and probes with high amplification efficiency at 37°C was screened successfully, and the concentration of reverse primer and the probe was 5μM, respectively. Furthermore, the RPA-nfo reaction was completed in 20 minutes at 37°C followed by a 3-5 minutes incubation at room temperature for development of an immunochromatographic strip.

Conclusions

All the results showed that the constructed RPA detection system has good specificity for detection of Coxiella burnetii without cross-reaction with other viruses, and can detect Coxiella burnetii at levels comparable to that of the quantitative PCR method.The
constructed RPA detection system showed superior detection performance, which could provide technical support for Coxiella burnetii in site detection.

FEMS7-0481
New Methods and Techniques

OPTICAL ELASTIC SCATTERING FOR LABEL-FREE IDENTIFICATION OF PATHOGENS

V. Genuer[1], D. Decq[1], J. Méteau[1], C. Belafdil[2], S. Benahmed[1], P.R. Marcoux[1], E. Schultz[1], O. Gal[2], M. Maurin[3]
[1]CEA, Division microTechnologies for Biology and Healthcare, 38054 Grenoble cedex 9, France
[2]CEA, DRT - LIST, 91191 Gif-sur-Yvette, France
[3]CHU Grenoble, Bacteriology Lab- CS10217, 38043 Grenoble cedex 9, France

Backgrounds

Screening *S. aureus* carriers on hospital admissions, especially before surgery, has been shown to reduce the occurrence rate of *S. aureus* infections. Given the large number of individuals requiring screening, rapid and cost-effective methods should be promoted. Chromogenic selective media such as ChromID *S. aureus* were introduced for this goal. They achieve the direct identification of *S. aureus* in one step, thus reducing workload and cost. However, they require a long incubation time (24h to 48h), and some confirmation tests in order to avoid false positive.

Objectives

We report here the ability of optical elastic scattering in discriminating *S. aureus* from other *staphylococci* at an early stage of growth (6h of incubation), directly on ChromID plate. Furthermore, it is compatible with any kind of confirmation tests as it is label-free, non-invasive and non-destructive.

Methods

Our instrument (called Microdiff) is based in Grenoble hospital and performs analyses of the scattering pattern (scatterogram) generated by a microcolony. A laser targets the microcolony to be identified and resulting photons are collected with a camera. Then pattern recognition algorithms yield in a few seconds the most probable identity for the probed microcolony.

Conclusions

A database of 4700 scatterograms over 34 strains was collected on microcolonies, at 6h of incubation on ChromID *S. aureus*. The obtained data were compared to a database so that machine learning can yield identification results. It gave a 91% discrimination rate between *S. aureus* and non-*aureus staphylococci*. As a comparison, color reading at 24h of incubation yields in average 93% of correct classification.

FEMS7-0826
New Methods and Techniques

MALDI-TOF SPECTRA ANALYSIS FOR DETECTION OF VANCOMYCIN-RESISTANT ENTEROCOCCUS FAECIUM

A. Mazzariol[1], *L. Galia*[1], *S. Dal Cero*[1], *A. Bertoncelli*[1]
[1]*University of Verona, Diagnostic and Public Health, Verona, Italy*

Backgrounds

Active screening with rectal swabs is useful to detect carriage and contribute to prevention of Vancomycin-resistant enterococci (VRE) healthcare-acquired infections

Objectives

Identify by Maldi-TOF characteristic peak for vancomycin resistance in *Enterococcus faecium*, to minimize reporting time and isolate carriers/patients as soon as possible.

Methods

The study included 196 clinical isolates of *E. faecium,* 140 of them were glycopeptides resistant strains obtained from rectal swabs during screening, and 56 were glycopeptides susceptible isolated from blood culture and urine.

All isolates were identified by Maldi-TOF Vitek MS (bioMerièux) with an adjusted protein extraction protocol, starting from a 1.5 McF solution and using 70% formic acid and equal volume of pure acetonitrile, to ncrease the Maldi-tof performance in the identification of *E. feciucm*

Vancomycin resistance determinants (*vanA, vanB, vanC*) were detected by PCR. All-resistant strains carried vanA, while susceptible strains didn't show any resistance determinant.

Good selected spectra of all strains were considered for analysis. Proteins common both susceptible and resistant strains and with a relative intensity lower than 0.03% were eliminated

We create a final super spectrum with all resistant strains that was compared with Enterococci database and identify the 7870 peak ± 4 Da that correlate with vancomycin resistance.

This peak was absent in all susceptible strains and present in 122 out 140 vancomycin-resistant showing a sensibility of 87% and a specificity of 100% in detecting vancomycin resistant strains**Conclusions**

The presence of the peak it will be useful in the rapid detection of vancomycin resistant clinical isolates.

FEMS7-0702
New Methods and Techniques

THE LEVELS OF TRACE ELEMENTS AND OXIDATIVE/NITROSATIVE STRESS PARAMETERS IN PATIENTS WITH BRUCELLA MELITENSIS

M. Miraloglu[1], E. Belge Kurutas[2]
[1], Adana, Turkey
[2]Faculty of Medicine- Sutcu Imam University, Department of Medical Biochemistry, Kahramanmaras, Turkey

Backgrounds

This is the first report regarding to investigate the activities of erythrocyte catalase (CAT) and superoxide dismutase (SOD), levels of plasma malondialdehyde (MDA), nitic oxide (NO), 3-Nitrotyrosine (3-NTx) as oxidative /nnitrosative stress parameters and serum zinc (Zn), copper (Cu) and selenium (Se) concentrations as trace elements were measured in patients with brucella melintensis, and results were compared with those of healthy individuals.

Objectives

The investigation included 31 patient with Brucella melitensis (age: 32.3±3.2) and 26 healthy subjects (age: 33.2±4.9) as control group who were admitted to Department of Medical Microbiology, Faculty of Medicine, Cukurova University.

Methods

The activities of erythrocyte CAT and SOD, levels of plasma MDA were measured as spectrophotometric. The levels of NO and 3-NTx in plasma were measured by ELISA. Serum Zn, Cu and Se concentrations were measured with flame atomic absorption spectrometry.

Conclusions

The mean of erythrocyte CAT and SOD activities and serum Zn and Se concentrations were significantly lower among patients compared with controls ($p<0.001$). However levels of plasma MDA in patients were comparable to controls and the mean NO, 3-NTx and levels in patients were significantly higher than controls ($p>0.001$). A significant positive correlation was found between levels of plasma MDA, NO, 3-NTx and serum Cu concentrations in patients with Brucella melitensis. Decreased Zn and Se levels, antioxidant system insufficiency and increased levels of MDA NO, 3-NTx and Cu were shown in patients with Brucella melitensis. Supplementary trace element antioxidative process may increase scavenger enzyme activities and also clinical symptoms may be amelioration in these patients.

FEMS7-1012
New Methods and Techniques

EFFECT OF THE YEAST COFLOCCULATION GENES FLO1, FLO5 AND FLO11 EXPRESSION ON THE YEAST-FILAMENTOUS FUNGUS CO-ADHESION IN A NOVEL IMMOBILIZATION METHOD, "YEASTS BIOCAPSULES"

J. Moreno García[1], F.J. Martín García[2], T. García Martínez[2], J.J. Moreno Vigara[3], J.C. García Mauricio[2], L.F. Bisson[4]
[1], Cordoba, Spain
[2]University of Córdoba, Microbiology, Córdoba, Spain
[3]University of Córdoba, Agricultural Chemistry, Córdoba, Spain
[4]University of California Davis, Viticulture and Enology, Davis, USA

Backgrounds

The use of immobilized systems for alcoholic fermentations offers many advantages over conventional free cell methods: facilitates higher cell densities, improves yield, allows the reutilization of the biocatalyst, among others. A novel method of yeast immobilization, called "yeasts biocapsules", has been developed in which yeast cells are attached to the hyphae of the fungus *Penicillium chrysogenum* forming a matrix in where substrates/products are easily diffused. Yeast cells and *P. chrysogenum* aggregate, or "coflocculate", to further form a biocapsule. Yeast genes *FLO1*, *FLO5* and *FLO11* have recently shown to be involved in the coflocculation process and may therefore be drivers of ecosystem organizational patterns.

Objectives

The aim is to analyze the impact of the yeast genes *FLO1*, *FLO5* and *FLO11* on the biocapsule formation.

Methods

Biocapsules were made with strains *S. cerevisiae* BY4742 and FY23 with *FLO* genes deletion and overexpression, the later constructed using *ADH2* and *HSP30* promoters.

Conclusions

Significant differences were found in terms of % yeast immobilized, with FY23 FLO11-ADH2 showing the highest values. Flo11p is a cell surface glycoprotein required for formation of fibrous interconnections between cells. These results shed light on yeast genes that influence yeast-fungus co-immobilization and might lead to an improvement of biocapsule yeast immobilization efficiency and further extend the field of application for this new system.

FEMS7-2648
New Methods and Techniques

EDEN: EVOLUTIONARY DYNAMICS WITHIN ENVIRONMENTS

P.C. Münch[1,2,3], *B. Stecher*[2,4], *A.C. McHardy*[1,3,5,6]
[1]*Helmholtz Centre for Infection Research, Computational Biology for Infection Research, Braunschweig, Germany*
[2]*Ludwig-Maximilian University of Munich, Max von Pettenkofer-Institute for Hygiene and Medical Microbiology, Munich, Germany*
[3]*German Centre for Infection Research DZIF, Partner Site Hanover-Brunswick, Brunswick, Germany*
[4]*German Centre for Infection Research DZIF, Partner Site LMU Munich, Munich, Germany*
[5]*Heinrich Heine University Dusseldorf, Cluster of Excellence on Plant Sciences, Dusseldorf, Germany*
[6]*Heinrich Heine University Dusseldorf, Department of Algorithmic Bioinformatics, Dusseldorf, Germany*

Backgrounds

Metagenomics revolutionized the field of microbial ecology, giving access to Gb-sized datasets of microbial communities under natural conditions. This enables fine-grained analyses of the functions of community members, studies of their association with phenotypes and environments, as well as of their microevolution and adaptation to changing environmental conditions.

Objectives

Phylogenetic methods for studying adaptation and evolutionary dynamics are not able to cope with big data. Calculating the dN/dS ratio for the large-scale sequence data sets that are being generated in metagenomics and comparative microbial genomics is very challenging, due excessive run times of current methods.

Methods

EDEN is the first software for the rapid detection of protein families and regions under positive selection, as well as their associated biological processes, from meta- and pangenome data. It provides an interactive result visualization for detailed comparative analyses. EDEN is available as a Docker installation under the GPL 3.0 license, allowing its use on common operating systems, at http://www.github.com/hzi-bifo/eden

Conclusions

We applied EDEN to 66 samples of the HMP project from six body sites sampled from healthy individuals. Across all body sites, most protein families with significant signs of positive selection in comparison to all other protein families were annotated with transport and binding functions, suggesting the existence of a functional pan-selectome. We also used EDEN to characterize human gut metagenome samples. EDEN determined a significantly higher dN/dS ratio for the protein coding genes from lean individuals compared to overweight and obese individuals, suggestive of a higher functional diversity in the guts of lean individuals.

FEMS7-2680
New Methods and Techniques

A METHOD FOR THE IN SILICO DETECTION OF PLASMID FRAGMENTS IN ENVIRONMENTAL SAMPLES

P.C. Münch[1,2,3], *B. Stecher*[2,4], *A.C. McHardy*[1,3,5,6]
[1]*Helmholtz Centre for Infection Research, Computational Biology for Infection Research, Braunschweig, Germany*
[2]*Ludwig-Maximilian University of Munich, Max von Pettenkofer-Institute for Hygiene and Clinical Microbiology, Munich, Germany*
[3]*German Centre for Infection Research, Partner Site Hanover-Brunswick, Brunswick, Germany*
[4]*German Centre for Infection Research, Partner Site LMU Munich, Munich, Germany*
[5]*Heinrich Heine University Dusseldorf, Cluster of Excellence on Plant Sciences CEPLAS, Dusseldorf, Germany*
[6]*Heinrich Heine University Dusseldorf, Department of Algorithmic Bioinformatics, Dusseldorf, Germany*

Backgrounds

Conjugation, the interbacterial transmission of circular DNA, is an biologically important and highly efficient mechanism for the horizontal spread of genes, allowing cells to adapt to different environments i.e. antibiotic resistance. With the aid of metagenomics – the application of random shotgun sequencing to environmental samples – we have the possibility to sequence whole microbial communities including bacterial chromosomes and conjugative genetic elements i.e. plasmid sequences.

Objectives

Until now, the extraction of the plasmid content from a metagenomic sample requires segregation steps which are complex, costly and only applicable to new experiments. Here, we propose a new method for the *in silico* identification and extraction of plasmid fragments allowing to study plasmid diversity and may improve assembly strategies.

Methods

We compared predictive performance of various learning strategies such as random forest (RF), support vector machine (SVM), relevance vector machines (RVM) and logistic regression for the classification task. In each case, we built a classifier based on sequence properties and k-mer content. Plasmidminer is available under the GPL 3.0 license at http://www.github.com/hzi-bifo/plasmidminer

Conclusions

We developed a software tool for discrimination between plasmid-derived and chromosome-derived sequences called Plasmidminer. The algorithm reaches an classification AUC ROC score of 0.93 (+/- 0.01) based on 10-fold cross validation for the discrimination of *Escherichia coli* chromosomes and their plasmid sequences. We furthermore plan to train the classifier to cope with all genomes that are available at NCBI and to handle read and contig-sized sequence fragments.

FEMS7-0443
New Methods and Techniques

THE FASTEST AVAILABLE METHOD TO DETECT SOMATIC COLIPHAGES, USED AS INDICATORS OF FECAL POLLUTION IN WATER AND FOOD

M. Muniesa[1], B. Elisenda[1], I. Lejla[1], P.B. Miriam[1], T. Daniel[1], L. Francisco[1], B. Anicet[1], J. Juan[1]
[1]Universitat de Barcelona, Genetic- Microbiology and Statistics, BARCELONA, Spain

Backgrounds

Somatic coliphages are indicators of fecal and viral pollution in water and food and one of the most reliable and cost effective methods. Several countries have already included them in their water management policies. Standardized methods (ISO, EPA) for their enumeration are available, but their implementation in routine laboratories will be favored if user-friendly commercial kits become available.

Objectives

Development of a new method to evaluate somatic coliphages, based in the use of a tailored *Escherichia coli* host strain that allows detection of <10 somatic coliphages in less than 3:00 h

Methods

The host strain *E. coli* WG5 was modified by replacing *uidB* and *uidC* genes encoding the transport of the glucuronic acid inside the cell, and overexpressing *uidA*, encoding the enzyme β-glucoronidase. Because the bacterial cell is unable to incorporate the substrate, the substrate only reaches the enzyme after the cellular lysis caused by phages. After phage-mediated lysis, the intracellular accumulated β-glucoronidase (overexpressed) release to the medium producing a change of color from yellow to dark blue.

Conclusions

The method is robust, sensitive (even less than 5 phages) and very fast. It requires only 1:30 h to detect 50 phages, and 2:15 h for 5 phages. The method is applicable to different types and volumes of environmental and food samples, liquid or solid. It can be also used for quantitative analysis applying the Most Probable Number (MPN). .

This is the fastest microbiological method detecting culturable fecal indicator microorganisms available so far.

FEMS7-1919
New Methods and Techniques

NEW RAPID PCR PROTOCOL BASED ON HIGH-RESOLUTION MELTING ANALYSIS (HRMA) TO IDENTIFY SACCHAROMYCES CEREVISIAE AND OTHER SPECIES WITHIN ITS GENUS

C. Nadai[1], *B. Bovo*[1], *A. Giacomini*[1,2], *C. Viviana*[1,2]
[1]*University of Padova, DAFNAE, Legnaro, Italy*
[2]*University of Padova, CIRVE, Conegliano, Italy*

Backgrounds

Selection projects aiming at the identification of new *Saccharomyces* strains are always on going as the use of the suitable yeast can strongly improve fermented food production, particularly winemaking. They are mainly targeted on *S. cerevisiae*, but others species in the *Saccharomyces* genus are of interest. For this reason, more and more efficient molecular techniques for yeast identification able to accelerate yeast selection process are always needed. Among the *Saccharomyces* genus, four yeasts are widespread in natural environments: S*. cerevisiae*, *S. bayanus*, *S. kudriavzevii* and *S. paradoxus*. Therefore, among the *Saccharomyces* species, their discrimination is of great interest.

Objectives

The development of an assay that allowed an easy, rapid and simultaneous discrimination among the *Saccharomyces* species during yeast selection programs.

Methods

A two-step protocol is proposed. Firstly the *Saccharomyces* genus identification is achieved by multiplex PCR analysis. Then, the *Saccharomyces* species is determined by a new method based on HRMA.

Conclusions

For HRMA two primer pairs have been proposed. The first was able to achieve the simultaneous identification of the four widespread *Saccharomyces* species, the second was used for the unambiguous discrimination of *S. cerevisiae* within its taxonomical genus.

FEMS7-0139
New Methods and Techniques

MCRAPD, POTENTIAL IMPORTANCE AND ITS POSITION IN THE TYPING OF BURKHOLDERIA MULTIVORANS AND BURKHOLDERIA CENOCEPACIA

L. Navrátilová[1], P. Jana[1], N. Radko[1], R. Vladislav[1]
[1]Palacký University Olomouc, Department of Microbiology, Olomouc, Czech Republic

Backgrounds

Typing techniques are extremely useful for nosocomial infection surveillance, their material and labour costs still limit their use to retrospective studies.

Objectives

We aimed to test the performance of techniques potentially useful for routine typing, namely the McRAPD.

Methods

48 bacterial isolates recovered from respiratory pathways from 19 patients with chronic pulmonary diseases from the Department of Respiratory Medicine and Department of Pediatrics, University Hospital Olomouc during the period from April 2012 to October 2016 and identified as *B. cenocepacia* or *B. multivorans* by MALDI-TOF MS. This set of isolates was expanded to include 3 epidemic strains of *B. multivorans* RAPD-III from University Hospital Olomouc, 3 reference strains from BCCM/LMG and the 4 strains of Motol University Hospital, which included the Czech and global epidemic strains. We used three RAPD primer AP-12, 270 and 272. For amplification was used LightCycler 96.

Conclusions

In *B. multivorans*, 4 patients were demonstrated to harbour a local hospital epidemic strain described earlier in patients under intensive care in our University Hospital, one of them had in addition another different strain. Whereas 3 patients had a unique strain. In *B. cenocepacia*, two patients had the Czech epidemic strain ST-32. All *B. cenocepacia* strains were different both from the global epidemic strain ET-12. All other strains were unique in individual patients.
McRAPD proved its applicability as rapid and economic strain typing technique. However, the universal applicability in different bacterial species makes this technique a promising screening tool for routine high-throughput epidemiological surveillance of infectious agents.

FEMS7-3226
New Methods and Techniques

OPTIMIZATION OF THE METHOD FOR EVALUATION OF THE STRINGENT RESPONSE AMONG DIFFICULT TO CULTIVATION BACTERIAL SPECIES

D. Nowicki[1], M. Cashel[2], A. Szalewska-Palasz[1]
[1]University of Gdansk, Bacterial Molecular Genetic, Gdansk, Poland
[2]National Institute of Child Health and Human Development, Molecular Genetics, Bethesda, USA

Backgrounds

The nucleotide second messengers, guanosine tetra- and penta- phosphate [(p)ppGpp], are responsible for the global downregulation of transcription, translation, DNA replication, and growth rate that occurs during the stringent response. More recent studies suggest that (p)ppGpp is also an important effector in many life processes, including virulence, persister cell formation, and biofilm production as well as antibiotic resistance.

Objectives

The aim of this work was to optimize a method of (p)ppGpp alarmone level assessment for clinical isolates and environmental strains.

Methods

We adapted ^{32}P nucleotide labeling method and thin layer chromatography separation on PEI cellulose plates to assess (p)ppGpp cellular level in strains that are difficult to cultivate. Specified, strain dependent cultivation conditions are one of the most problematic steps in stringent response evaluation for many bacterial isolates. In the routinely used isotope labeling technique cells are grown in defined MOPS minimal medium to avoid influence of pH variation and salinity on TLC separation. Our assay is fitted for most of poorly culturable strains as *Enterococcus faecalis*, *Streptococcus epidermidis*, *Listeria monocytogenes*, *Shigella sonnei*, *Staphylococcus aureus*. The cultures are pre-grown overnight on defined medium plates and then are shifted to liquid medium for ^{32}P labeling for 30'. We used 96-well plate format to rapid and efficient determination of alarmone induction which makes this methods suitable also for screening tests in response to specific stress agents including antibiotics.

Conclusions

The presented method may be useful to assess the stringent control of metabolism in clinical isolates, environmental strains, or other slow growing bacteria.

FEMS7-1469
New Methods and Techniques

ANTIBIOFILM ACTIVITY OF THE THIN ZINC OXIDE FILM FORMED BY ATOMIC LAYER DEPOSITION UNDER UV-A LIGHT

K.H. Park[1], M. Antoinette[1], G.D. Han[2], H.J. Kang[1], J.H. Shim[2], H.D. Park[1]
[1]Korea University, Civil- Environmental and Architectural Engineering, Seoul, Republic of Korea
[2]Korea University, Mechanical Engineering, Seoul, Republic of Korea

Backgrounds

Microorganisms tend to aggregate and adhere to a surface by forming biofilms. Because biofilm cells are embedded by extracellular polymeric substances secreted by themselves, they are less sensitive to antibiotics or antimicrobial agents than planktonic counterpart. Synthesis of a surface resistant to biofilm formation is an important issue, especially when a surface comes in contact with water. One approach for synthesizing an antibiofilm surface is to provide it ability to produce reactive oxygen species (ROS).

Objectives

To synthesize an antibiofilm surface, we formed zinc oxide (ZnO) thin films on glass slides by atomic layer deposition (ALD). Although ZnO nanoparticles are reported to produce ROS, it has not been verified whether ZnO films formed by ALD would produce ROS and, in turn, inhibit biofilm formation. Therefore, this study was aimed to quantify ROS production and evaluate antibiofilm activity by the ZnO film.

Methods

Nanostructures of the ZnO films were characterized by X-ray diffraction, atomic force microscopy, and field-emission scanning electron microscopy. Photocatalytic activities of the ZnO films were analyzed by measuring ROS including superoxide anion, hydroxyl radical, and singlet oxygen. *Staphylococcus aureus* biofilms were formed on the ZnO films in a drip-flow device. Antibiofilm activity was evaluated by confocal laser scanning microscopy.

Conclusions

The ZnO films consisted of closely packed nano-sized hexagonal wurtzite crystalline structures. Under UV-A light irradiation, they produced ROS actively, comparable to ZnO nanoparticles. More importantly, the ZnO films could reduce *S. aureus* biofilms more than half, suggesting that the ZnO thin films formed by ALD is an effective antibiofilm surfaces.

FEMS7-0407
New Methods and Techniques

OPTIMAL RNA EXTRACTION METHOD FOR THE ANALYSIS OF BACTERIAL SMALL RNAS CONTAINED IN MEMBRANE VESICLES

C. Garcia-Bonillo[1], C. Perez-Cruz[1], E. Mercade[1]
[1]University of Barcelona, Department of Biology- Healthcare and the Environment, Barcelona, Spain

Backgrounds

Membrane vesicles (MVs) are released by many Gram-negative bacteria and *Pseudomonas aeruginosa* is one of them. Currently, the presence of *small* RNA (sRNAs) in MVs is being extensively analysed in eukaryotes but remains nearly unexplored in prokaryotes. The methodology of extraction of sRNAs may influence the results obtained in further studies.

Objectives

To determine the optimal RNA extraction method for the analysis of bacterial *s*RNAs contained in MVs in order to support future studies aimed at determining the biological roles of these sRNAs.

Methods

Three different RNA extraction methods were assayed: miRCURY™ RNA isolation kit – Cell & Plant content (EXIQON®), FastRNA™ SPIN Kit for Microbes (MP®) and miRNeasy Mini Kit (QIAGEN®). These kits were used to extract total RNA and, quality and purity of extracted RNA was determined by OD values, capillary electrophoresis and fluorimetry. The efficiency of extraction of *P. aeruginosa* PAO1 *s*RNAs (RsmZ, CrcZ and PhrS) was studied by RT-qPCR.

Conclusions

Results indicate that the kits EXIQON® and MP® extract more effectively the *small* RNAs fraction than QIAGEN®. EXIQON® is recommended to extract *small* RNAs from MVs because the obtained RNA profiles are cleaner, the protocol is easier to perform and the number of copies does not present significant differences with MP®. As the different RNA isolations methods give extensive variations in the sRNAs yields and patterns, it is crucial to select an RNA isolation approach depending on the research purpose.

FEMS7-0828
New Methods and Techniques

EXPLODING AN INTERNAL BOMB. BACTERIAL TOXIN-ANTITOXIN SYSTEMS AS A TARGET FOR NOVEL ANTIMICROBIALS

M. Równicki[1,2], T. Pieńko[2,3], J.I. Castilo Silva[2,4], D. Bartosik[5], J. Trylska[2]
[1]College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland
[2]Centre of New Technologies, University of Warsaw, Warsaw, Poland
[3]Faculty of Pharmacy with the Laboratory Medicine Division, Medical University of Warsaw, Warsaw, Poland
[4]Colegio "La Paz", A.C. La Paz S/N, Tijuana- B.C.-, Mexico
[5]Institute of Microbiology, University of Warsaw, Warsaw, Poland

Backgrounds

Insufficient progress in tackling antibiotic resistance has made the development of novel antimicrobial strategies a prime concern. The bacterial toxin–antitoxin systems (TAs) are modules that play a crucial role in bacterial persistence, pathogenicity and cell survival due to the presence of an "internal bomb" – toxin.

Objectives

In this study we investigated an artificial activation of TA using antisense peptide nucleic acid (PNA) oligomers. We targeted TA *mazEF* that was found ubiquitous in *Enterobacteriaceae*.

Methods

The secondary structure of mRNA antitoxin (*mazE*) was predicted using the RNAfold and Mfold software. On this basis, we selected specific regions as targets for antisense inhibition by complementary oligonucleotides. We synthesized PNA (anti-*mazE*) with the cell penetrating peptide $(KFF)_3K$ to assure the delivery of PNA through bacterial cell walls. To evaluate the antimicrobial effectiveness of such PNA we determined the minimal inhibitory concentration (MIC) for bacterial cultures of enteropathogenic *E. coli*. By performing quantitative reverse transcript (qRT)-PCR we examined the decay of *mazE* mRNA in viable bacterial cells after treatment with PNA at different concentrations. Additionally, we checked synergistic interactions between anti-*mazE* PNA and selected antibiotics (polymyxin B, trimethoprim, sulfamethoxazole) defining fractional inhibitory concentration (FIC). Moreover, we determined the population of persister cells after treatment with PNA and with PNA in combination with antibiotics.

Conclusions

Our findings showed that TA *mazEF* could become a novel target for developing antibacterial compounds. We believe that TAs should be investigated further as an effective antibacterial strategy against bacteria.

FEMS7-0848
New Methods and Techniques

VITAMIN B12 AS A NOVEL CARRIER FOR ANTISENSE PEPTIDE NUCLEIC ACIDS TO BACTERIAL CELLS

M. Równicki[1,2], M. Wojciechowska[2], A. Wierzba[3], J. Czarnecki[4], D. Bartosik[4], D. Gryko[3], J. Trylska[2]
[1]College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland
[2]Centre of New Technologies, University of Warsaw, Warsaw, Poland
[3]Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland
[4]Institute of Microbiology, University of Warsaw, Warsaw, Poland

Backgrounds

The widespread emergence of bacterial resistance to known antibiotics creates a need to develop novel antibacterials. The use of short, modified oligonucleotides as inhibitors of bacterial transcription or translation seems a promising strategy. However, the main and yet unsolved problem precluding their use as antibiotics is that bacteria do not uptake synthetic oligonucleotides from the environment.

Objectives

We examined vitamin B12 (cobalamin) as a natural carrier for peptide nucleic acid (PNA) oligomers into Gram-negative *Escherichia coli* and *Salmonella* Typhimurium cells.

Methods

To provide a convenient system to monitor the effect of antisense PNA in *E. coli* and *S.* Typhimurium, we constructed a reporter vector expressing red fluorescent protein (RFP) optimized for expression in *Enterobacteriaceae*. We designed and synthesised an antisense PNA sequence (anti-*rfp* PNA) targeted at mRNA of the Red Fluorescence Protein (RFP). To evaluate the potential of antisense PNA to inhibit the production of RFP, we grew bacteria in the presence of anti-*rfp* PNA covalently linked to five different vitamin B_{12} derivatives. As a control we used PNA conjugated to the most widely used cell-penetrating peptide $(KFF)_3K$. We examined different types and lengths of the spacer between vitamin B_{12} and PNA, including a cleavable disulfide bond. We found that vitamin B_{12} transports antisense PNA to Gram – negative cells more efficiently than the $(KFF)_3K$ peptide. We also found that the structure of the linker impacts the antisense effect.

Conclusions

Our study provides the foundation for developing vitamin B_{12} as a carrier for PNA oligonucleotides to bacterial cells for any desired applications.

FEMS7-1073
New Methods and Techniques

DEVELOPMENT OF A MALDI IMAGING MASS SPECTROMETRY APPROACH TO BACTERIAL PROTEOMICS: FIRST APPLICATION TO LISTERIA MONOCYTOGENES BIOFILMS EXPOSED TO DESICCATION

T. Santos[1], D. Centeno[2], C. Chambon[2], D. Viala[2], M. Hébraud[1,2]
[1]INRA, Université Clermont Auvergne- INRA- UMR MEDiS, Saint-Genès-Champanelle, France
[2]INRA, Plateforme d'Exploration du Métabolisme PFEM, Saint-Genès-Champanelle, France

Backgrounds

Human listeriosis cases are due to the ingestion of contaminated foods with *Listeria monocytogenes* and most cases are connected with food contamination in industries. The control of *L. monocytogenes* is difficult to achieve in processing environments due to its survival capabilities. Water availability has particular biological importance and bacteria are submitted to variations in air relative humidity (RH) in food processing plants. This bacterium is also able to grow as a biofilm but the underline features as how biofilms adapt to desiccation are not well-known. Matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS) is a surface-sampling technology that can determine spatial information and relative abundance of analytes directly from biological samples. Spectra are collected and each peak intensity in the spectra is used to generate an ion intensity map.

Objectives

This study aims to develop an IMS approach to explore the protein expression and *in situ* distribution of proteins within *L. monocytogenes* biofilms exposed to desiccation.

Methods

L. monocytogenes biofilms were grown in MCDB medium during 48h before being exposed to a moderate desiccation environment (24h at 10ºC/ 75% RH), mimicking the food workshop conditions. After matrix spraying, mass spectra were acquired on a MALDI-TOF/TOF MS and processed through SCiLS software.

Conclusions

Data analyses allowed to distinguish protein localization patterns between the two conditions and chose target mass peaks for further analysis. These data demonstrate how imaging can be used to dissect the spatial proteome of an intact bacterial biofilm giving a new insight into protein regulation relating to biofilm adaptation.

FEMS7-2645
New Methods and Techniques

DEVELOPMENT OF A STRAIN-SPECIFIC REAL-TIME PCR ASSAY FOR ENUMERATION OF A PROBIOTIC LACTOBACILLUS REUTERI IN CHICKEN FEED AND INTESTINE

V.A. Sattler[1], *M. Mohnl*[1], *V. Klose*[1]
[1]*BIOMIN Research Center, TFZ, Tulln, Austria*

Backgrounds

Strain-specific identification of probiotics is essential for accurate quantification and monitoring of the bacteria in animal feeding studies.

Objectives

Objective was to develop a strain-specific real-time PCR assay for quantification of a probiotic *Lactobacillus reuteri* (DSM 16350) in poultry feed and intestine.

Methods

Strain-specific primers pwere designed from *L. reuteri* DSM 16350 genomic sequences derived after suppression subtractive hybridization with the type strain *L. reuteri* DSM 20016. Specificity was tested using a set of non-target strains. Applicability of the real-time PCR assay was evaluated in a controlled broiler feeding trial. The probiotic *L. reuteri* was quantified in feed from three feeding phases and in intestinal samples of the jejunum, ileum, and caecum of three, 14, and 39 day old birds. In all probiotic supplemented feed *L. reuteri* was enumerated close to the inclusion rate of 7.0 x 10^3 cfu/g, and was not detected in control feed. In three day old birds *L. reuteri* DSM 16350 was only detected in intestinal samples from probiotic fed animals ranging from 8.2 ± 7.8 x 10^5 cfu/g in the jejunum, 1.0 ± 1.1 x 10^7 cfu/g in the ileum, and 2.5 ± 5.7 x 10^5 cfu/g in the caecum. Similar results were obtained for intestinal samples of older birds. With increasing age of birds the amount of *L. reuteri* signals in the control animals also increased.

Conclusions

This strain-specific real-time PCR assay could be used to assure accurate inclusion of the probiotic to the feed and to monitor it’s uptake into the GIT of young chicken.

FEMS7-2006
New Methods and Techniques

EXPLORING NOVEL ANTIMICROBIAL AGENTS FROM GUT METAGENOME

V. Sharma[1], A. Sharma[1], S. Kumar[1], S. Gupta[1]
[1]Indian Institute of Science Education and Research Bhopal, Biological Sciences, Bhopal, India

Backgrounds

The efficacy of antibiotics against bacterial infections is decreasing, thus there is a need to search for potential alternatives to antibiotics.

Objectives

Two novel antibacterial agents, enzymes and peptides, appear promising for treating bacterial infections. Among these, Peptidoglycan hydrolases can be used as alternate antibacterial agents due to their unique property of cleaving peptidoglycan cell wall present in both gram-positive and gram-negative bacteria. Small peptides can be used as anti-microbial agents due to their potential as inhibitors of biofilm.

Methods

Based on the site of action of peptidoglycan hydrolases, a Random Forest-based computational tool 'HyPe' (http://metagenomics.iiserb.ac.in/hype/) is developed for identification and classification of novel peptidoglycan hydrolases from genomic and metagenomic data. It displayed 71.12%-100% sensitivity, 99.98% specificity, 99.55% accuracy and 0.80-0.94 MCC values, and thus can be used to predict novel antibacterial agents. Similarly, to predict the anti-microbial nature of small peptides on biofilms, we have developed a unique computational method ‘BioFin’ (http://metagenomics.iiserb.ac.in/biofin/) based on Support Vector Machine-based prediction models and additionally by including sequence motifs information. It displayed the > 97% accuracy and > 0.84 Matthews Correlation Coefficient (MCC) values and thus, can be used to predict antimicrobial peptides.

Conclusions

HyPe can be used for the identification and classification of novel antibacterial peptidoglycan hydrolases and BioFin can be used as a tool for the prediction of antibacterial peptides from complete genomic/metagenomic ORFs. The results and highlights of these two novel antibacterial agent prediction tools will be presented.

FEMS7-0241
New Methods and Techniques

APPLICATION OF FUNGAL LACCASES FOR OXIDATION OF HYALURONIC ACID

D. Smirnou[1], M. Hermannová[2], L. Franke[1], J. Jílková[1], S. Pepeliaev[1], V. Velebný[3]
[1]Contirpo a.s., R&D, Dolni Dobrouc, Czech Republic
[2]Contipro.a.s., R&D, Dolni Dobrouc, Czech Republic
[3]Contirpo a.s., CEO, Dolni Dobrouc, Czech Republic

Backgrounds

Hyaluronan (HA) is a high-molecular glycosaminoglycan, which fulfils important functions in human organism. Due to unique properties and biocompatibility, HA is abundantly used in medicine and cosmetics. Extra functionality is added to HA by means of modification of its chemical structure. Introduction of aldehyde groups in HA molecule is one of the most required modifications. Our study shows that this reaction can be performed by applying fungal enzymes laccases.

Objectives

To develop a laccase-mediated process of primary hydroxyl on *N*-acetyl-D-glucose amine oxidation to aldehyde.

Methods

HA oxidation with enzyme-mediator system, composed of laccase from fungi *Trametes versicolor*, *Pleurotus ostreatus* or *Agaricus bisporus*, and mediators TEMPO, 4-amino-TEMPO or 4-acetamido-TEMPO was studied. For the best enzyme-mediator system reaction parameters were optimized. Chemical structure of oxidized HA was characterized using NMR and LC-MS.

Conclusions

“Green” technology for HA oxidation using enzyme-mediator system was elaborated. The reaction was conducted in mild conditions without application of highly corrosive reagents. Primary hydroxyl on C6 of *N*-acetyl-D-glucose amine in HA molecule was oxidized highly specifically. For oxidized HA with degree of substitution to aldehyde 7 – 12 %, over-oxidation to carboxyl was as high as 2.8% only. HA oxidation applying fungal enzymes laccases is an advantageous technology over the conventionally used TEMPO/NaOCl technique.

FEMS7-2001
New Methods and Techniques

CELLS-PMAQPCR ASSAY FOR DIRECT AND RAPID QUANTIFICATION OF SACCHAROMYCES CEREVISIAE VIABLE CELLS IN WINE

V. Soares-Santos[1], *P. Borredat*[1], *I. Pardo*[1], *S. Ferrer*[1]
[1]*ERIBiotecMed, ENOLAB, Valencia- Burjassot, Spain*

Backgrounds

Saccharomyces cerevisiae is the main species involved in alcoholic fermentation. Despite its main role in winemaking, *S. cerevisiae* is able to spoil sweet wines, causing refermentations. Molecular methods, as quantitative PCR (qPCR), have been developed for rapid detection and enumeration of yeasts. Recently, some dyes as propidium monoazide (PMA), have been used in conjunction with qPCR to selectively detect live cells. Nonetheless, this methodology requires a previous DNA extraction step that greatly increases the overall assay time and cost.

Objectives

To develop a fast and reliable qPCR method in conjunction with PMA, and without DNA extraction steps (Cells-PMAqPCR). To apply the developed methodology to detect and quantify viable cells of *S. cerevisiae* in white and red wines.

Methods

Optimization of cells suspensions treatment by different amounts of PMA (0, 5, 10, 25, 50, 100, 200 µM). Construction of standard curves in culture medium, white and red wines matrices. Comparison of the Cells-PMAqPCR assay with plate count values.

Conclusions

The developed method allows differentiating between live and dead cells of *S. cerevisiae* both in white and red wines, avoiding DNA extraction and overcoming the presence of inhibitors like polyphenols and ethanol. Cells quantification was linear over five orders of magnitude and detected as few as one viable cell per reaction in all the matrices. The Cells-PMAqCells provides a useful tool for the specific, fast and reliable quantification of viable cells, enabling winemakers to take quick decisions controlling the viability of the starters, the fermentation process, and the risk of refermentation.

FEMS7-2015
New Methods and Techniques

LAMP ASSAY FOR DIRECT AND RAPID DETECTION OF OENOCOCCUS OENI CELLS IN WINE

V. Soares-Santos[1], *I. Pardo*[2], *S. Ferrer*[2]
[1]*Universitat de Valencia, Departamento de Microbiologia y Ecologia, Valencia- Burjassot, Spain*
[2]*ERIBiotecMed, ENOLAB, BURJASSOT, Spain*

Backgrounds

Oenococcus oeni is the most common species of lactic acid bacteria associated with the malolactic fermentation (MLF) in wine. Fast enumeration of *O. oeni* is necessary to determine whether MLF is likely to be performed or not, and to decide if the use of a commercial starter is needed. Traditional methods for enumerating microorganisms involve time-consuming plating and microscopic observation techniques, which means delays in taking wine-processing decisions. Recently, a novel molecular method termed loop-mediated isothermal amplification (LAMP) allows fast amplification of DNA with high specificity, sensitivity under isothermal amplification condition.

Objectives

To design new primers and adapt the LAMP methodology to detect *O. oeni* directly in white and red grape musts and wines.

Methods

New *O. oeni* LAMP specific primers have been designed, and their specificity has been evaluated. LAMP reactions have been performed in culture media, and white and red grape musts and wines.

Conclusions

A designed primer set allowed the specific detection of *O. oeni* by LAMP in the culture medium, and in white and red grape must and wine matrices, overcoming the presence of inhibitors as ethanol and polyphenols. The detection limit was 12 and 120 cells per reaction in white and red wines, respectively. The LAMP assay for the identification of *O. oeni* is advantageous in terms of speed (<40 min), specificity and ease of operation compared with traditional methods. Only a thermostatic isothermal bath or block is required for a visual detection of microorganisms, and can be easily adapted for a specific quantification.

FEMS7-2247
New Methods and Techniques

SWINE UROTHELIUM CELL CULTURE – A NEW MODEL FOR ASSESSING UROPATHOGENIC POTENTIAL OF HUMAN UPEC STRAINS

L. Predojević[1], D. Žgur-Bertok[1], D. Keše[2], M. Erdani Kreft[3], M. Starčič Erjavec[1]
[1]University of Ljubljana - Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia
[2]University of Ljubljana- Faculty of Medicine, Institute of Microbiology and Immunology, Ljubljana, Slovenia
[3]University of Ljubljana- Faculty of Medicine, Institute of Cell Biology, Ljubljana, Slovenia

Backgrounds

Escherichia coli (*E. coli*) is a member of the gut microbiota however, *E. coli* strains capable of causing intra and extraintestinal infections do exist. A prominent group of pathogenic *E. coli* strains are uropathogenic *E. coli* strains (UPEC).

Objectives

The main aim of this study was to set up a model for evaluation of the uropathogenic potential of *E. coli* strains based on normal urothelial cell cultures derived from swine bladders.

Methods

The following *E. coli* strains were used: UPEC strains (J96 and 536), two laboratory strains (MG1655 and DH5α) and a commensal strain (BJ16) isolated from the feces of a healthy human. The following methods were used: cultivation of cell cultures in wells on plastic plates, preparation of bacterial suspensions, pathogenicity assays with determination of cell viability using the trypan blue staining method. On the basis of viability (percent of live cell culture cells) the pathogenic potential of tested *E. coli* strains was determined.

Conclusions

Viability of urothelium cells after 1h exposure to *E. coli* strains J96, 536, MG1655, DH5α and BJ16 was 85%, 94%, 83%, 88%, and 92%, respectively. The viability of urothelium cells after a 3h exposure to the employed *E. coli* strains was 53%, 73%, 91%, 95% and 89% (in the same order as above). The obtained results clearly demonstrate that the swine urothelium cell culture can be used as a model for human UPEC.

FEMS7-1959
New Methods and Techniques

MYCROBIOTA: A USER-FRIENDLY GALAXY APPLICATION FOR MICROBIOTA DETERMINATION AND DYNAMIC REPORTING FROM 16S RRNA GENE SEQUENCES

A. Stubbs[1], S. Boers[2], S. Hiltemann[1], B. Kriesels[1], P. van der Spek[1], R. Jansen[3], J. Hays[2]
[1]Erasmus MC, Bioinformatics, Rottedam, The Netherlands
[2]Erasmus MC, MMIZ, Rottedam, The Netherlands
[3]Streeklab, Molecular biology, Haarlem, The Netherlands

Backgrounds

Many researchers have embraced 16S rRNA gene profiling techniques that has led to a wealth of publications and documented differences in the composition of microbial communities. The resultant microbiota profiles are generated using bioinformatics approaches for which sophisticated software, such as mothur and QIIME, are freely available. Unfortunately however, the use of these bioinformatics tools remains complex for the non-bioinformatics scientist.

Objectives

Development of MYcrobiota – a standardized analytical workflow to determine bacterial taxa from 16s rRNA gene sequences using the functionality enabled within Galaxy.

Methods

Galaxy is a user-friendly web tool, which provides access to complex bioinformatics tools and workflows via a simple graphical user interface (GUI). We have implement mothur as a set of ~100 Galaxy tools to deliver 16S rRNA gene analysis. These tools have been combined as an “end to end” analysis service (MYcrobiota) for one or multiple samples with user defined reporting based on our iReport functionality [Hilteman et al 2015]. The associated iReport includes a dynamic visualization of bacterial taxonomies – from phylum to genus level detected per sample and a means to summarize the whole experiment using the dynamic Phinch metagenomics viewer integrated into Galaxy. We demonstrate the functionality of MYcrobiota using a published 16S rRNA gene dataset from Boers, Hays and Jansen (2015).

Conclusions

The mothur Galaxy tool set are available for download from the Galaxy toolshed (https://toolshed.g2.bx.psu.edu/) and for use with MYcrobiota at https://bioinf-ttt.erasmusmc.nl/MMIZgalaxy. The utility of our mothur Galaxy toolset is demonstrated with our online tutorial (https://github.com/shiltemann/training-material/blob/add-metagenomics-module/Metagenomics/tutorials/MOTHUR-MiSeq-SOP.md) based on the mothur MiSeq SOP.

FEMS7-1918
New Methods and Techniques

OBTENTION OF MINIMUM INHIBITORY CONCENTRATION (MIC) PREDICTION EQUATIONS FOR ANTIBACTERIAL QUINOLONES VERSUS PROTEUS MIRABILLIS AND MORAXELLA CATARRHALIS

B. Suay[1], J.I. Bueso-Bordils[1], M.T. Perez-Gracia[1], G. Anton-Fos[1], L. Lahuerta-Zamora[1], R. Martin-Algarra[1], P. Aleman-Lopez[1]
[1]University CEU Cardenal-Herrera, Pharmacy, Valencia, Spain

Backgrounds

The search of new molecules with therapeutic activity is a laborious process with an elevated economic cost. Quantitative relationships between chemical structure and activity have meant an important saving of money and time. Among the different methods used, molecular topology has showed to be a useful tool to find new drugs.

Objectives

In this work, a multilinear regression analysis was carried out in order to look for functions capable of accurately predicting biological properties of a group of quinolones.

Methods

The functions were developed with data regarding quinolone activity due to their fast evolution and pharmacological properties. The species targeted in this study were *Proteus mirabillis* and *Moraxella catarrhalis* because these Gram-negative bacteria are very common human pathogens. The studied properties were minimum inhibitory concentration 90 (MIC_{90}) versus these two microorganisms. Structural description was achieved through topological indices. Randomization and cross-validation by using leave-one-out test were also performed in order to assess the stability and the prediction ability of the functions selected.

Conclusions

The following prediction equations were obtained with good statistical data:

$$MIC_{90}Pm = -2.58 - 84.04^{6}\chi ch - 8.11^{3}\kappa + 36.93 IShannon$$

(n=14; SEE=1,487; SEE(cv)=1.866; r^2=0.92; r^2(cv)= 0.858)

$$MIC_{90}Mc = -2.83 - 0.103^{6}\chi_p + 0.987 NI_2 + 1.6 J_4$$

(n=13; SEE=0.018; SEE(cv)=0.033; r^2=0.884; r^2(cv)=0.637)

The obtained results demonstrate that molecular topology is a very useful tool in the prediction of microbiological properties.

FEMS7-0931
New Methods and Techniques

BACTERIAL CELL MEMBRANE BARCODING, A SERS MAPPING METHODOLOGY FOR IDENTIFICATION AND DETECTION OF POTENTIAL PATHOGENIC BACTERIA

T. Szoke-Nagy[1,2], N.E. Dina[1], A. Colniță[1], I.B. Cozar[1]
[1]National Institute for Research and Development of Isotopic and Molecular Technologies, Department of Molecular and Biomolecular Physics, Cluj-Napoca, Romania
[2]Babes-Bolyai University - Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, Cluj-Napoca, Romania

Backgrounds

To develop a new technique for rapidly identification and detection of potential pathogenic microorganisms, using an ultrasensitive method based on Surface-enhanced Raman spectroscopy (SERS), as alternative to conventional culture-based and nucleic acid-based methods.

Objectives

Determination of SERS marker bands for identification of potential pathogenic microorganisms, isolated from the environment.
Preliminary tests for identification of bacteria from liquid media that mimic the complex biological sample.

Methods

Bacterial strains were isolated from environmental samples and were identified based on 16S rRNA molecular markers, using 27FB-1492R primer pair.
The SERS spectra was recorded with a confocal Renishaw inVia Reflex Raman Spectrometer using either the 532nm (Cobolt, Diode Pumped Solid State – DPSS – 200 mW) or 633 nm (He-Ne laser – 17 mW) excitation line, by using the 100× objective (Leica, NA 0.9, WD 3.4 mm). The SERS-active silver clusters were generated by using the *in situ* synthesis, in the presence of the bacterial biomass. The SERS fingerprinting is based on enhanced Raman signal arising from the “hot-spots” generated in the close proximity of the bacterial cell wall.
The bovine serum was used as complex biological media; prior to use, bovine sera was centrifuged using Vivaspin® 15R Centrifugal Concentrator in order to remove the serum proteins.

Conclusions

Specific SERS marker bands were identified for both Gram-positive and Gram-negative strains, specific for bacterial cell membrane components. By using robust chemometric data analysis tools, the discrimination at strain level of bacteria was assessed; even predicting their virulence or resistance to drug treatment is possible.

FEMS7-3055
New Methods and Techniques

THE MULTI LOCUS SEQUENCE TYPING (MLST) OF PASTEURELLA MULTOCIDA, A TECHNICAL APPROACH

K. Tadayon[1], R. Ghgaderi[1], A.R. Jabbari[1], M. Sekhavati[1], A. sanchooli[1], A. arab[1], R. Banihashemi[1]
[1]Razi Vaccine & Serum Research Institute- Agricultural Research- Education and Ex, Aerobic Bacterial Vaccine, Karaj, Iran

Backgrounds

Currently, there are two *Pasteurella multocida* MLST schemes including the British Multiple host MLST that holds isolates from variety of animal hosts, including cattle, sheep, pigs and birds. This database is more concentrated on evolutionary relationships between the isolates. The second database is Australian and was developed by the Rural Industries Research and Development Corporation so-called RIRDC MLST with an initial focus on avian isolates and epidemiological theme.Both systems assay polymorphism in limited stretches of 7 housekeeping genes among which four are shared by both.

Objectives

The fact that there is no previous MLST experience on *Pasteurella multocida* in Iran, a principle scope of the present work was establishment of the technique.

Methods

Using the Primer 3 program, seven new primer pairs recommended by RIRDC MLST were designed against the genome of P. multocida P70 reference strain. This was performed in a way that 7 PCR products matching with the corresponding target sections of the of adk, est, gdh, mdh, pgi, pmi and zwf genes were all within the range of 500-700 bp.

Conclusions

Amplification protocols were optimized and set as a single identical protocol could be used for all the 7 loci. Feasibility of these modifications was successfully tested against a single field isolate and two vaccinal strains of *Pasteurella multocida.*Our observations proved suitability of the employed modifications that can be easily used in any MLST work on *Pasteurella multocida*

FEMS7-0145
New Methods and Techniques

NOVEL DERIVATIVE OF ARYLIDENIMINO-1,3-PYRIMIDINES (MET-253) AS POTENTIAL DRUG CANDIDATES FOR THE TREATMENT OF PNEUMOCYSTIS PNEUMONIA

V. Tetz[1], *T. George*[2]
[1]*TGV-inhalonix- LLC, Microbiology, New York, USA*
[2]*TGV-inhalonix- Inc, Microbiology, New York, USA*

Backgrounds

Pneumocystis pneumonia is a life-threatening lung disease caused by fungus *Pneumocystis* spp. PCP is still a frequent and severe opportunistic infections in immunocompromised patients. Treatment of PCP remains a challenge due to high mutation rates of *Pneumocystis* spp. to standard compounds and treatment-associated toxicity. The goal was to examine in vitro MICs and LD50 of the novel antifungal "Met-253", against *P.carnii* and *P.murina*

Objectives

Both pentamidine and Met-253 (first-in-class antifungal, derivative of arylidenimino-1,3-pyrimidines (C12H8Cl2N3NaO3) were found to display antimicrobial activity anti-Pneumocystis activity (Tables 1 and 2).

Table 1. Average Met-253 IC50 Determinations

Fungi	24h (mcg/ml)	48h	72h
P.carnii	3.90+/-2.0	2.56+/-0.57	1.61+/-1.72
P.murina	3.30+/-0.19	1.50+/-0.13	0.165+/-0.06

Table 2. Percent reduction in ATP/media control

Fungi	Drug (mcg/ml)	24h	48h	72h
P.carnii	Pentamidine 1	81.14	86.55	87.57
	Met-253 10	68.2	90.27	95.58
	Met-253 1	10.2	12.78	26.78
P.murina	Pentamidine 1	92.07	97.7	98.12
	Met-253 10	76.56	98.51	97.92
	Met-253 1	10.82	42.11	94.42

Met-253 demonstrated moderate activity against *P.carnii* and marked activity in *P.murina*. Reduction in ATP occurred in a time and dose-dependent manner over the 72-h testing period LD50 values of Met-253 in mice were 7980±750mg/kg (p.o.) and 13880±920mg/kg (p.o.) in rats.

Methods

P.carnii and *P.murina*, were distributed into 48-well-plates with final concentration of 7.5log10nuclei/ml Pc anf 6.5log10nuclei/ml Pm. Control and compound dilutions were added and

incubated at 36^{O}C, 5%CO2. At 24-48-72h 10% of the well volume was removed and the ATP content was measured using luciferin-luciferase assay. IC50 was calculated using INSTAT linear regression program. Triplicate IC50 determinations were averaged. Oral LD50 of Met-253 in was determined. Experiments were executed in accordance with the Guide for the Care and Use of Laboratory. LD_{50} was calculated according to the method described by Litchfield and Wilcoxon.

Conclusions

Our study (some data were generated by NIH/NIAID) demonstrated that novel antifungal Met-253 possesses high anti-Pneumocystis activity and low toxicity.

FEMS7-2081
New Methods and Techniques

UV AND MASS SPECTROMETRY IMAGING METABOLOMICS FOR THE DECONVOLUTION OF MICROBIAL INTERACTIONS.

V. Gonzalez-Menendez[1], R. Serrano[1], F. Muñoz[2], J. Martin[2], G. Martinez[1], O. Genilloud[1], J.R. Tormo[2]
[1]Fundacion MEDINA, Microbiology, Granada, Spain
[2]Fundacion MEDINA, Chemistry, Granada, Spain

Backgrounds

Co-culturing has proved to be an effective tool to simulate the physiological conditions that occur during microbial interaction in their natural environment and may have an enormous potential for the discovery of new molecules with therapeutic applications. Recent detection techniques that evaluate natural products by Image Mass Spectrometry (IMS) have been shown to be extremely suitable for analyzing microbial interactions and detecting in situ activation of cryptic pathways.

Objectives

Develop a methodology to visually map the secondary metabolites produced by microorganisms and identify those induced by the interactive co-cultivation.

Methods

In order to provide a spatial dimension to the microbial interaction, samples were analyzed by uHPLC-UV and by low resolution mass spectrometry (LR-MS) in the range of positive m/z for each extract. Mass spectra were collected from 150 m/z to 1500 m/z in positive mode. Compound management and automated micro-extraction methodologies were combined with in house HPLC Studio 2.0 and MASS Studio 1.0 software developments to generate ultraviolet and mass spectrometry images of interacting co-cultured endophytes.

Conclusions

After a screening of interesting co-cultures this technology was applied on two clear antagonic strains in order to set up this characterization methodology and their metabolomics interactions. Data analyses highlighted patterns of localized induction of secondary metabolites, otherwise not produced when strains were cultured axenically, and proved that this technology can speed up the discovery of induced natural products from on microbial interactions.

FEMS7-2998
New Methods and Techniques

BACTERIOPHAGE AS EFFECTIVE DECOLONIZING AGENT FOR STAPHYLOCOCCUS AUREUS CARRIERS

A. Ulu Kilic[1], A. Gundogdu[2], H. Kilic[3], B. Aslan[4], E. Alp[1]
[1]Erciyes University, Infectious Diseases and Clinical Microbiology, Kayseri, Turkey
[2]Erciyes University- Faculty of Medicine-, Microbiology and Clinical Microbiology, Kayseri, Turkey
[3]Erciyes University Medical Faculty, Microbiology and Clinical Microbiology, Kayseri, Turkey
[4]Erciyes University, Microbiology, Kayseri, Turkey

Backgrounds

Staphylococcus aureus (SA) is a common cause of hospital- and community-acquired infections. Asymptomatic colonization with SA in the nares may precede infection. There is great interest in preventing the transmission of SA and decolonizing persons who harbor these bacteria.

Objectives

We aimed to investigate invitro activity of multiple lytic phage cocktails as decolonising agent.

Methods

This study included *SA* strains isolated from nasal swabs of hospitalized patients and blood culture of bacteremic patients. Identification of strains was performed using conventional microbiological methods. The in vitro susceptibilities of SA isolates obtained from colonized and bacteremic adult patients examined by using six different commercial phage preparations. These were Pyo-bacteriophage (Pyo), Intesti-bacteriophage (intesti), Enko-phage (Enko), SES-bacteriophage (Ses), Fersisi-bacteriophage and Staphylococcal bacteriophage (Sb). Susceptibility of isolates was determined by observing confluent, semi-confluent, opaque lysis or individual plaques. The absence of any lysis has been reported as resistance.

Conclusions

Forty two MRSA strains isolated from blood culture of bacteremic patients were included to the study. Of them 5 (12%) found resistant to phage cocktails for Pyo, 9 (21%) for SES, 10 (23.8%) for Fersisi, 3 (7%) for İntesti, 10 (24%) for Enko, 6 (14.2%) for Sb. Only one of these strains was resistant to all six phage cocktails.Twenty eight isolates obtained from nasal swabs of patients routinely screened for colonization by *SA* were included to the study. Of all, among methicilin sensitive isolates only one (1/25, 4%) was resistant to all six phage cocktails. Of all 3 MRSA strains one was resistant to all phage cocktails. Bacteriophages may be considered as effective decolonizing agent for SA nasal carriers. For this approach to be successful in clinical settings, need to study a cocktail of phages covering a larger spectrum of strains is required.

FEMS7-2747
New Methods and Techniques

AN INTEGRATIVE PROTEOGENOMICS STRATEGY TO IDENTIFY THE ENTIRE PROTEIN CODING POTENTIAL OF PROKARYOTIC GENOMES

A. Varadarajan[1,2], U. Omasits[1], S. Goetze[2], M. Robinson[3], B. Wollsceid[2], C. Ahrens[1]
[1]Agroscope & SIB Swiss Institute of Bioinformatics,
Research Group Molecular Diagnostics- Genomics and Bioinformatics, Wadenswil, Switzerland
[2]ETH Zurich,
Department of Health Sciences and Technology & Institute of Molecular Systems Biology & BioMedical Proteomics Platform BMPP, Zurich, Switzerland
[3]University of Zurich, Institute for Molecular Life Sciences & SIB Swiss Institute of Bioinformatics, Zurich, Switzerland

Backgrounds

Accurate genome annotation is relevant at all levels, from small, focused experiments to large functional screens, systems biology studies, upto prediction of gene regulatory, interaction and metabolic networks. Yet, the over-prediction of random ORFs and underrepresentation of true, functional sORFs (short ORFs) represent a major limitation.

Objectives

We present a proteogenomics strategy that integrates protein-coding genes from major reference genome annotation resources, gene prediction algorithms and *in silico* ORFs. Using this approach, we create minimally redundant integrated proteogenomics databases (iPtgxDBs) that cover the entire protein-coding potential of prokaryotic genomes.

Methods

Data from a complete expressed proteome searched against the iPtgxDB helped to uncover novel ORFs, start sites and incorrectly predicted pseudogenes including cases uniquely predicted by each resource. We show that the E-value distributions of PSMs for novel hits and target proteins is similar, and confirm expression of novel ORFs and expressed pseudogenes by parallel reaction monitoring (PRM).

Conclusions

Our approach is generic (it worked for genomes with widely varying GC content), and flexible: iPtgxDBs can be created for key model organisms (e.g. E.coli BW25113, parental strain of the Keio-knockout collection) up to newly sequenced genomes. We illustrate this with expression evidence for ORFs in unique genome regions of a lab strain compared to its NCBI reference genome, down to even single amino acid variations, which will have implications in clinical proteomics and beyond. We will release iPtgxDBs for several organisms to enable a large number of research groups to apply proteogenomics in the initial genome annotation step.

FEMS7-2958
New Methods and Techniques

DETECTION AND QUANTIFICATION OF ML01, A GENETICALLY MODIFIED SACCHAROMYCES CEREVISIAE WINE STRAIN, USING EVENT-SPECIFIC METHOD BASED ON QUANTITATIVE PCR

E. Vaudano[1], *A. Costantini*[1], *E. Garcia-Moruno*[1]
[1]*CREA-Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria -, microbiology, Asti, Italy*

Backgrounds

Despite the availability of transgenic wine yeast for several years, their use is only authorized in a few countries and limited to two strains: ML01, able to convert malic acid into lactic acid during alcoholic fermentation, and ECMo01 suitable for reducing the risk of carbamate production. In the remaining countries which produce wine, to date, their use has not been allowed.

Objectives

In this work we propose an event-specific qPCR technique based on the use of specific primers and probes for the detection and quantification of a transgene event in GM ML01 *Saccharomyces cerevisiae* in relation to the *S. cerevisiae* taxon-specific target *MRP2*. The applicability of the technique has been tested for determination of the GM ML01 strain in active dry yeast preparation.

Methods

The method consists in efficient extraction of DNA and qPCR (quantitative PCR) analysis based on event-specific assay targeting *MLC* (malolactic cassette), and a taxon-specific *S. cerevisiae* assay detecting the *MRP2* gene.

Conclusions

The ADY DNA extraction methodology has been shown to provide good purity DNA suitable for subsequent qPCR. The *MLC* and *MRP2* qPCR assays showed characteristics of specificity, dynamic range, limit of quantification (LOQ) limit of detection (LOD), precision and trueness, which were fully compliant with international reference guidelines. The method has been shown to reliably detect 0.005% (mass/mass) of GM ML01 *S. cerevisiae* in commercial preparations of ADY.

FEMS7-2007
New Methods and Techniques

EVALUATING OGATAEA POLYMORPHA MALTASE SUBSTRATE SPECIFICITY VIA CLASSICAL ACTIVITY-BASED ASSAYS AND A NOVEL DSF METHOD

K. Viigand[1], *K. Ernits*[1], *K. Põšnograjeva*[1], *T. Visnapuu*[1], *T. Alamäe*[1]
[1]*University of Tartu, Genetics, Tartu, Estonia*

Backgrounds

Maltases (EC 3.2.1.20) and isomaltases (EC 3.2.1.10) are α-glucosidases releasing glucose from a set of α-glucosidic substrates and belong to family 13 of glycoside hydrolases (GH13) according to CAZy classification. These enzymes are hypothesized to have evolved from an ancestral promiscuous α-glucosidase ancMALS through gene duplications and mutations as described in Voordeckers *et al*. (2012). *S. cerevisiae* maltases use maltose-like sugars whereas isomaltases use isomaltose-like ones. Resurrected ancMALS used both. We are studying methylotrophic yeast *Ogataea (Hansenula) polymorpha* which has diverged early from the yeast main line of evolution and is considered a "phylogenetically old" yeast. MAL1 has extended substrate specificity and its properties are highly similar to those of hypothetical ancMALS.

Objectives

To introduce the methods for evaluation of substrate specificity of α-glucosidases using MAL1 of *O. polymorpha* and a variety of α-glucosidic sugars.

Methods

Two activity-based methods (including a microplate assay) using monitoring of glucose release from various α-glucosidic substrates by the wild-type MAL1 and a differential scanning fluorimetry (DSF) of a catalytically inactive mutant of MAL1 in the presence and absence of α-glucosidic substrates are characterized.

Conclusions

A high-throughput DSF method allows to predict substrate selection pattern of the MAL1. The DSF results were in good accordance with substrate specificity data obtained from the activity-based assay. A cost-efficient microplate-based activity assay was successfully employed.

Acknowledgements: This work was financed by ERC grant GLTMR1050P (PUT1050).

References

Voordeckers K., Brown C.A., Vanneste K., *et al*. 2012. *PLoS Biol.* **10**: e1001446.

FEMS7-2677
New Methods and Techniques

ALIVE OR DEAD - LIMITS OF ENUMERATION

L. Vondrakova[1], J. Pazlarova[1], K. Demnerova[1]
[1]University of Chemistry and Technology Prague, Department of Biochemistry and Microbiology, Prague 6, Czech Republic

Backgrounds

Molecular-genetic methods based on nucleic acid detection are indisputably very reliable and fast tools used to identify various pathogens that pose a threat to public health. However, main disadvantage of these methods is the inability to distinguish signals originating from different sources (live, dead and compromised cells or free DNA) which is significant limitation especially when used for quantification of live infective agents.

Objectives

The aim of this study was to perform comprehensive research and comparison of methods applicable when precise quantification of pathogens is desirable. Since the definition of “dead cell” in microbiology is rather delicate, several techniques based on different approach were included.

Methods

Taking into account the most common criteria of cell viability determination, the multiplex qPCR in combination with various sample pre-treatment approaches was introduced. Two DNA-intercalating dyes - ethidium monoazide and propidium monoazide (membrane integrity), flotation (cell buoyant density), and flow cytometry (metabolic activity) were examined. In addition, one of the most important variable of cell life - its death, was included in the study. Since the mechanism of cell destruction can strongly influence the effectivity and result interpretation of abovementioned sample pre-treatments, several different strategies of cell inactivation were applied (UV, peracetic acid, hydrogen peroxide, cold plasma, autoclaving etc.).

Conclusions

Reliable method for identification and quantification of three most abundant thermotolerant campylobacters in the form of multiplex qPCR was designed. Impact of various sample pre-treatment approaches and possible errors in order to distinguish the origin of detected DNA was evaluated.

FEMS7-0211
New Methods and Techniques

A PROBE-FREE AND SENSITIVE DETECTION METHOD FOR HUMAN DIARRHEA-CAUSING PATHOGENS USING QUADRIPLEX RT-PCR COMBINED HIGH RESOLUTION MELTING ANALYSIS

H. Wang[1], Q. Mo[1], Z. Yang[1], Q. Wang[1], C. Tu[1], J. Lin[1]
[1]Zhuhai International Travel Healthcare Center, State Key Laboratory of Diarrhea Disease Detection, Zhuhai, China

Backgrounds

Diarrhea is one of the most common public health issues worldwide. Rapid and sensitive diagnostic methods are urgently needed to help physicians make faster and better treatment decisions for those patients who suffered from diarrhea.

Objectives

To establish a probe-free quadriplex RT-PCR combined high resolution melting analysis (HRMA) assay for the rapid and sensitive detection of four major diarrhea-causing pathogens, including rotaviruses, astroviruses, noroviruses and sapoviruses.

Methods

Specific primers and amplification parameters of the probe-free quadriplex RT-PCR as well as the range and ramp of melting temperature in the HRMA were optimized to establish the assay. Specificity and sensitivity of the assay were analyzed and compared with conventional RT-PCR. Clinical stool samples were used to evaluate the efficacy of the new assay.

Conclusions

After several rounds of optimization, the quadriplex RT-PCR combined HRMA assay was established successfully with high specificity. Sensitivity analysis indicated that the lower limit of detection were 10^0, 10^2, 10^0 and 10^3 copies/reaction for rotaviruses, astroviruses, noroviruses and sapoviruses, respectively, which were 1000-fold, 10-fold, 1000-fold and 10-fold more sensitive than conventional RT-PCR. Clinical evaluation showed that the assay was 100% concordant to conventional RT-PCR, indicating the high reliability of the new assay.To the best of our knowledge, this is the first quadriplex RT-PCR combined HRMA assay established for the detection of four major diarrhea-causing viruses. The assay would provide a valuable platform for the probe-free, rapid and sensitive diagnosis of these pathogens.

FEMS7-0130
New Methods and Techniques

HIGH-CONTENT SCREENING OF MORPHOLOGICAL CHANGES IN RESPONSE TO ANTIBIOTIC TREATMENT

T. Zahir[1], R. Camacho[2], J. Hofkens[2], J. Vermant[3], M. Fauvart[1], J. Michiels[1]
[1]KU Leuven, Bioscience Engineering, Leuven, Belgium
[2]KU Leuven, Molecular Imaging and Photonics, Leuven, Belgium
[3]ETH Zurich, Materials, Zurich, Switzerland

Backgrounds

High-content screening is a powerful and emerging method in molecular biology. Automated microscopy, sophisticated image analysis algorithms and genome-scale knock-out/knock-down libraries are increasingly being used for functional genetics, genetic profiling and for building genetic networks.

Objectives

Genome-wide screening of mutant phenotypes upon antibiotic treatment is the aim of many studies. One particularly daunting challenge in this regard is the high-throughput observation of morphological changes in individual bacteria over time. Here, we use automated microscopy for time-resolved measurement of responses to antibiotics at the single-cell level.

Methods

We have developed a high-content screening method using automated phase-contrast microscopy and image analysis for the purpose of high-throughput morphology-based studies in bacteria. As a case study, we measured dynamic responses (morphological changes and killing kinetics) of mutants in all non-essential genes of *Escherichia coli* after treatment with β-lactam antibiotics – cefsulodin, cephalexin and mecillinam.

Conclusions

The screen resulted in a comprehensive image dataset characterizing all mutants' response to inhibition of different penicillin-binding proteins. Using image-based cell counts, we identified mutants with altered killing kinetics. Mutants that showed atypical cell morphologies were identified by implementing machine learning algorithms. These results not only reveal genetic determinants of bacterial drug tolerance and susceptibility but also indicate roles of in cell envelope biogenesis. The high-content screening method will enable the study of dynamic phenotypes, hitherto restricted to low-throughput analysis techniques, on an unprecedented scale, promising new and important insights into mechanisms of antibiotic activity and gene function.

FEMS7-0512
New Methods and Techniques

SPECTRA PATTERN MATCHING WITH IN SILICO HYPOTHESIS VALIDATION FOR BACTERIAL TYPING

Y. Zhu[1]
[1]*Ecole Polytechnique Federale de Lausanne, Institut of chemical sciences and engineering, Sion- Valais, Switzerland*

Backgrounds

Rapid and reliable microbial identification is crucial for effective antibiotics therapy and reduction of public health cost. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) profiling of bacteria has been exploited for quick bacterial identification since 1990s, based on spectra pattern matching between sample spectra and a bank of standard bacterial spectra (*i.e.* a database). The algorithm used for pattern matching and the database are important for the success of this MS-based identification.

Objectives

We aim to develop an effective algorithm for mass spectra pattern matching that can be used for bacterial typing.

Methods

A four-step approach is included in the new algorithm: *1)* a raw sample mass spectrum is pre-processed to generate a peak-list with mass-to-charge ratio, normalized intensity, and full width at half maximum; *2)* three similarity scoring methods, *i.e.* relative Euclidean distance, relative Euclidean distance weighted by peak intensity, and Cosine correlation, are applied to calculate the similarity score between the sample spectrum and each reference spectrum in a database based on peak-lists; *3) in silico* random spectra model is used for calculation of false discovery rate ; and *4) in silico* similar spectra model is adopted for calculation of P-value (the ratio of similar spectra with similarity scores against the combined spectrum lower than the similarity score between the combined spectrum and the matched reference spectrum).

Conclusions

A new algorithm was developed for MALDI-MS based bacterial typing. According, a web-based application at http://bacteriams.fudan.edu.cn/ is built and is open freely to public usage.

FEMS7-0477
New Methods and Techniques

ELECTROPORATION-MEDIATED INACTIVATION OF C. LUSITANIAE CELLS AND PSEUDOHYPHAE

A. Zinkeviciene[1], E. Lastauskiene[2], V. Novickij[3], A. Grainys[3], I. Girkontaite[1], A. Paskevicius[4], J. Svediene[4], S. Markovskaja[5], J. Novickij[3]
[1]State Research Institute Centre for Innovative Medicine, Immunology, Vilnius, Lithuania
[2]Vilnius University, Microbiology and Biotechnology, Vilnius, Lithuania
[3]Vilnius Gediminas Technical University, Institute of High Magnetic Fields, Vilnius, Lithuania
[4]Nature Research Centre, Laboratory of Biodeterioration Research, Vilnius, Lithuania
[5]Nature Research Centre, Laboratory of Mycology, Vilnius, Lithuania

Backgrounds

Antifungal resistance is a growing concern worldwide and research for the alternative methods for the biocontrol of *Candida* yeasts is systematically performed. *Candida lusitaniae* is unique among *Candida* species due to its quick development of resistance to amphotericin B and antifungal resistance-associated phenotypic switching, which allows rapid adaptation to changing environment. As a result alternative non-chemical methods for biocontrol of Candida-associated surface infections are of particular interest, one of which could be electroporation. Electroporation utilizes high pulsed electric fields for induction of cell membrane permeability changes and thus increasing molecular transport. It is safe for use on human and already covers a wide range of applications in biotechnology, food processing and biomedicine.

Objectives

Investigate the feasibility of electroporation for inactivation *C. lusitaniae* cellular and pseudohyphae phenotypes and determine the threshold electric field for high permeabilization of yeast.

Methods

Electroporation protocol – square wave pulse 100 µs x 8, 2.5–25 kV/cm. Permeabilization was evaluated using propidium iodide assay (50 µM) by flow cytometry using Amnis FlowSight system at 550 nm wavelength. Cell viability assay was performed by plating cells after electroporation on the YPD medium and counting CFU. Amphotericin B susceptibility assay was performed by microdilution and agar diffusion methods.

Conclusions

We showed that 2.5 – 25 kV/cm electric field pulses of a fixed duration (100 µs x 8) induce a dose dependent permeabilization of yeast. Pseudohyphae morphology yeasts with the 25 times increased resistance to amphotericin B can be effectively inactivated after applying pulsed electric field higher than 15 kV/cm.

FEMS7-2073
New Methods and Techniques

DNA STRUCTURAL ALIGNMENT ALGORITHM CAN PREDICT PLASMID MOBILITY AND HOST RANGE BY LOCATING DNA SUBSTRATES FOR PLASMID TRANSFER

J. Zrimec[1], *A. Lapanje*[2]
[1]*Faculty of Health Sciences, Computational biology and bioinformatics, Izola, Slovenia*
[2]*Institute Jozef Stefan, Department for Environmental Sciences, Ljubljana, Slovenia*

Backgrounds

Due to the increasing problem of antimicrobial resistance, modelling and analysis of horizontal gene transfer processes is an essential research area. We focus on the most widespread and harmful of these processes, conjugation, where transfer of plasmids between different microbial hosts allows the storage and transfer of resistance genes.

Objectives

I will present a new approach to analysis of plasmids based on interpreting the origin-of-transfer, a merely 200 bp long non-coding plasmid DNA region that is the enzymatic substrate for the relaxase. Using a statistical approach we have only recently verified that these enzymatic DNA substrates contain conserved structural properties in relation to groups of conserved relaxases, and that computational DNA structure prediction much more accurately distinguishes substrate properties than analysis of nucleotide sequences.

Methods

Using a newly developed structural representation of DNA and bioinformatic algorithms, we developed a procedure to determine the mobility of plasmids, which can predict even the range of potential plasmid hosts. The procedure is based on structural alignment algorithms that proved effective in uncovering new DNA substrates. Based on an initial set of 64 experimentally determined transfer substrates we have identified hundreds of new ones.

Conclusions

The tools for prediction of DNA structural properties and for determining the mobility and host range of plasmids were implemented as web tools on the server http://dnatools.eu. The algorithm for structural alignment of DNA enables characterisation of a number of new DNA substrates and regulatory regions that until now could not be detected using existing methods.

FEMS7-2211
New Methods and Techniques

A SIMPLE AND CONVENIENT METHOD TO ASSESS THE DIVERSITY OF METAGENOMIC SAMPLES BASED ON PCR AMPLIFICATION OF 16S RRNA

J. Zrimec[1], *T. Rijavec*[2], *A. Lapanje*[2]
[1]*Faculty of Health Sciences, Computational biology and bioinformatics, Izola, Slovenia*
[2]*Institute Jozef Stefan, Department of Environmental Sciences, Ljubljana, Slovenia*

Backgrounds

Metagenomic analyses are crucial to study the diversity, activity and dynamics of uncultivable microbes. An integral part in the analyses is the polymerase chain reaction (PCR), which can present a bottleneck due to PCR errors, such as when amplifying parts of 16S rRNA genes.

Objectives

Often in agarose electrophoresis gels we see smeared DNA bands of wrong size, which are considered to be non-specific PCR errors and are eliminated. However, if these amplicons are not errors, then a part of the sample's true diversity is lost. Therefore, we explore if band smearing after PCR is in fact caused by imperfectly paired strands of the amplified DNA.

Methods

We used synthetic oligonucleotides to mimic 16S rRNA variable regions in PCR and analyse how smear in agarose gels and 16S rRNA sequence heterogeneity are related. DNA structures from separate band and smear gel fractions were extracted using a new electroelution procedure, sequenced, and analysed with bioinformatic tools to show correctly and imperfectly paired DNA strands.

Conclusions

When amplifying highly heterogeneous target DNA, such as 16S rRNA, imperfect pairing of the amplified DNA can lead to band smearing in agarose gels, which is not an indicator of low specificity of the PCR, but in fact, of sample diversity. Since the smear in agarose gels is only a structural part of the correctly amplified DNA, it carries important information about the richness and diversity of the analysed microbial communities. Quantifying the amount of smear can be a useful method to assess the diversity of metagenomic samples.

FEMS7-0626
Pathogens / Pathogenicity

SILVER NANOPARTICLES SYNTHESIS FROM DRACOCEPHALUM MOLDAVICA AS NEW CANDIDATE FOR ANTIBACTERIAL ACTIVITY

H. Abbaspour[1], Z. Haghighi Pak[1], N. Karim[2], A. Fattahi[3]
[1]Islamic Azad University, Biology, Damghan, Iran
[2]Razi University, Biology, Kermanshah, Iran
[3]kermanshah University, Pharmaceutics, kermanshah, Iran

Backgrounds

Nowadays, scientists are going to after finding a way to control and prevent many diseases by new biomaterial with the lowest side effects. From scientific point of view, silver nanoparticle extracted from plants such as *Dracocephalum moldavica* is the matter of importantce. Since this nanoparticle have many advantages such as cost effective, ability to adaptation to environment, antibacterial and antifungal effects, it can be used as a good candidate for medical care.

Objectives

In this study extraction of silver nanoparticle from *D. moldavica* and its antibacterial effects were investigated.

Methods

Dracocephalum moldavica seeds were purchased from Pakan Bazr Isfehan. Green synthesis method was done to synthesise of nano-silver from mentioned plant.To confirm this synthesis, some analysis such as EDX,TFIR,XRD and TEM were done. Antibacterial effects of silver nanoparticle on *Staphylococcus aureus*, *Staphylococcus epidermidis, Esherichia coli and pseudomonas aeruginosa* were investigated by well diffusion method and minimal inhibitory concentration were determined too.

Conclusions

The results showed that silver nanoparticle extracted from *D.moldavica* had antimicrobial effect. The zone of Inhibition growth of *Staphylococcus aureus*, *Staphylococcus epidermidis ,Esherichia coli and pseudomonas aeruginosa* in the present of nano-silver were obtained,8/5mm,11mm,17/5mm and 13 mm, respectively. The best minimal inhibitory concentration was gained 11µg ml^{-1} for *E.coli*.

Knowledge-wise *D.moldavica* is one of the beneficial plants and this study showed that nano-silver extracted from this plant had antibacterial effect, so this plant's extraction can be a proper candidate tool to treat and control disease in near future.

FEMS7-2685
Pathogens / Pathogenicity

THE SERINOPROTEASE PIC FROM ESCHERICHIA COLI INDUCES AN INTENSE INFLAMMATORY RESPONSE BY M1 MACROPHAGES IMMUNOMODULATION

Y.A. Lima[1], I.L. Dutra[2], J.R. Nascimento[2], A.A.M. Vale[2], E.M. Sousa[3], R.M. Silva[4], W.P. Elias[5], F.R.F. Nascimento[2], A.G. Abreu[2,3]
[1]Federal University of Maranhão, Laboratory of Immunophysiology, São Luís, Brazil
[2]Federal University of Maranhão, Programa de Pós-Graduação em Ciências da Saúde, SÃO LUIS, Brazil
[3]CEUMA University, Programa de Pós-Graduação em Biologia Parasitária, São Luís, Brazil
[4]Federal University of São Paulo, Departament of Microbiology- Immunology and Parasitology, São Paulo, Brazil
[5]Instituto Butantan, Laboratory of Bacteriology, São Paulo, Brazil

Backgrounds

Some *Escherichia coli* strains are important pathogens responsible for a variety disease, and albeit using distinct mechanisms of pathogenesis, have in common the production of Pic. We have recently shown that Pic mediates immune evasion by the direct cleavage of complement molecules, significantly reducing complement activation by all three pathways.

Objectives

The aim of this study was to investigate the action of Pic on macrophages immunomodulation.

Methods

The ability of bacteria to induce macrophage polarization to M1 or M2 (pro-inflammatory and anti-inflammatory profile, respectively), as well as the cytokines and nitric oxide (NO) production were evaluated after infection of RAW264.7 macrophages. Macrophages were incubated with Pic-producing *E. coli* (F5), F5Δ*pic* mutant or purified Pic for 6, 12 and 24 h. Immunophenotyping was performed using specific antibodies and analyzed by flow cytometry. **Results:** Six hours after infection, an increase in NO production was observed in all groups infected with bacteria, being statistically significant in the cells infected with *E. coli* F5. Interestingly, purified Pic induced an intense NO production, as well as the cytokines IL-6, MCP-1 and TNF-α. On the other hand, there was no statistically significant IL-10 production among groups. In addition, the immunophenotyping also showed a high expression of pro-inflammatory markers (IaIe and LyC6) on the cells infected by Pic or Pic-producing bacteria.

Conclusions

Pic has a high potential to induce M1 macrophage polarization with intense inflammatory response. Evaluation of this mechanism in animal model is underway to better understand the role of Pic in the context of sepsis.

FEMS7-2204
Pathogens / Pathogenicity

A SNAPSHOT OF THE MULTIDRUG-RESISTANT MYCOBACTERIUM TUBERCULOSIS STRAINS IN LIMA, PERU, AS A FIRST STEP TO TAILOR MOLECULAR TOOLS TO FAST TRACK HIGH-RISK CIRCULANT STRAINS

F. Acosta[1,2], J. Agapito[3], M. Herranz[1], F. Barletta[3], E. Abascal[1], L. Otero[3], G. Romero[3], C. Sola[4], E. Bouza[1], E. Gotuzzo[3], D. Garcia de Viedma[1]
[1]Instituto de Investigación Sanitaria Gregorio Marañón- Hospital General Universitario Gregorio Mara ñón- CIBERES- CIBER Enfermedades Respiratorias, Servicio de Microbiología, Madrid, Spain
[2]Universidad de Alcalá, Departamento de Biomedicina y Biotecnología., Madrid, Spain
[3]Instituto de Medicina Tropical Alexander von Humboldt- Universidad Peruana Cayetano Heredia, TB Research Unit, Lima, Peru
[4]Institute for Integrative Biology of the Cell I2BC-, CEA- CNRS- Univ. Paris-Sud- Université Paris-Saclay, F-91405 Orsay-Cedex, France

Backgrounds

Multidrug-resistant tuberculosis (MDR-TB) has become a major health problem. According to the WHO-TB report, cure rates for MDR-TB are lower (50%-70%). The high rates of MDR-TB in Peru, demands the implementation of more effective epidemiology strategies. Among them, to tailor strain-specific PCRs targeting high-risk strains could mean a new strategy in controlling MDR-TB.

Objectives

To describe the transmission clusters of MDR-TB in Lima, looking for hot-spot transmission areas where specific MDR strains are more actively spread.

Methods

We genotyped by MIRU-VNTR (24-loci) all the consecutive MDR-TB cases in 2014-15 from the 32 health centers of San Juan de Lurigancho district (North-Lima). MIRU-VNTR patterns were analyzed using MIRU-VNTRplus to assign lineages and BioNumerics to identify transmission clusters.
Among 59 MDR-MTB isolates (around 20% of the total MDR cases), the most prevalent sublineages were LAM (L4.3; 62.7%) and Haarlem (L4.1.2; 22.0%). The Beijing lineage (L2) was identified in 6.7% of the isolates. 34 isolates (57.6 %) were included in 9 transmission clusters (2-9 isolates). Five of these clusters, including one involving a Beijing strain, corresponded to strains that had already been identified in a previous study of 51 MDR strains isolated in the same district in 2011.

Conclusions

Genotyping of MDR-TB allowed us to identify high-risk strains actively transmitted in localized settings in Lima. These data allow to activate the design of tailored strain-specific PCRs to fast track their detection and optimize the control of their transmission.

Financial support: Programa de Becas IFARHU-SENACYT (270-2016-293). ISCIII (FIS15/01554) and ERA-Net LAC (ELAC2015/T08-0664).

FEMS7-1923
Pathogens / Pathogenicity

CLONAL OUTBREAKS OF [PASTEURELLA] PNEUMOTROPICA BIOVAR HEYL IN TWO MICE COLONIES

S. Adhikary[1], H. Christensen[1], M. Bisgaard[2], F. Dagnæs-Hansen[3]
[1]University of Copenhagen, Department of Veterinary Disease Biology, Frederiksberg C, Denmark
[2]University of Copenhagen, Emeritus, Sjælland, Denmark
[3]Aarhus University, Department of Biomedicine, Aarhus, Denmark

Backgrounds

[*Pasteurella*] *pneumotropica* is generally considered as an opportunistic pathogen in rodents. The bracket indicates that the bacterium is not a true member of the family *Pasteurellaceae*. It is classified as two biovars, Jawetz and Heyl. In [*P.*] *pneumotropica* infections, clinical diseases have mainly been associated with the respiratory tract of rodents. Furthermore, mastitis, orbital, urogenital and subcutaneous infections have also been reported. However, these reports have not referred the specific biovar of [*P.*] *pneumotropica.* This study investigated the association of biovar Heyl with the outbreak strains that are related to mastitis, orbital, cutaneous and vaginal abscesses.

Objectives

To document that [*P.*] *pneumotropica* biovar Heyl is pathogenic and can cause clonal outbreaks in mice.

Methods

Fifty three isolates from two mice colonies were studied. Primary identification was done by colony morphology and 'classical' biochemical test. To investigate clonal relationship, Pulsed-field gel electrophoresis (PFGE) was performed and assessed by cluster analysis. Five strains were selected for partial sequencing of the *rpoB* gene to represent the clusters. For comparison with outbreak strains, the epidemiologically unrelated six strains were also included.

Conclusions

The isolates were identified as [*P.*] *pneumotropica* biovar Heyl based on phenotype and genotype. PFGE analysis resulted in five related profiles that differed in one to four fragments, considered as the same outbreak clone according to 'Tenover criteria'. The outbreak strains were diverged from the epidemiologically unrelated strains. Therefore, this natural outbreak of different clinical conditions caused by the same clone provides evidence of the pathogenicity of [*P.*] *pneumotropica* biovar Heyl.

FEMS7-3263
Pathogens / Pathogenicity

ANTIMICROBIAL ACTIVITY AGAINST MYCOBACTERIUM TUBERCULOSIS UNDER IN VITRO DORMANCY CONDITIONS

D.A. Aguilar Ayala[1,2], S. Rivera Gutiérrez[1], J.F. Cerna Cortés[1], J.C. Palomino[2], P. Vandamme[2], J.A. González y Merchand[1], A. Martin[3]
[1]ENCB - IPN, Microbiología, Ciudad de México, Mexico
[2]Ghent University, Biochemistry and Microbiology, Gent, Belgium
[3]Institute of Experimental and Clinical Research, Université Catholique de Louvain, Brussels, Belgium

Backgrounds

Although tuberculosis (TB) treatment is dependent on the implementation of drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (*Mtb*) clinical isolates, and molecular detection of gene mutations associated with drug resistance, treatment failure remains a challenge for better controlling TB. Some studies have suggested that treatment failure is probably due to the survival of dormant mycobacteria. Additionally, host lipids have shown an important role during the dormant stage as well as during resuscitation of *Mtb*.

Objectives

The objective of this study was to evaluate in a lipid-rich dormancy model, the susceptibility of *Mtb* to two known anti-TB drug combinations: rifampicin, moxifloxacin, amikacin and metronidazole (R-MX-A-MZ), and R-MX-A plus pretomanid (PA).

Methods

Mtb H37Rv was cultured in the presence of a mixture of cholesterol and fatty acids, until reaching exponential and stationary phases of growth as well as NRP1 and NRP2 hypoxic stages of dormancy. Once the mycobacteria reached each experimental phase, the mixture of antibiotics, R-MX-A-MZ or R-MX-A-PA, was added. The antibiotic activity was evaluated at 7, 14 and 21 days by estimating mycobacterial survival in solid and liquid media.

Conclusions

Our results show for the first time that the presence of lipids as carbon source may confer *Mtb* some tolerance against the mixture of antibiotics tested, and this tolerance could be even higher during dormant stages. Since current DST are based on aerobic cultures with dextrose as carbon source, implementation of a DST in clinical strains that includes lipids as carbon source could potentially lead to a better treatment strategy.

FEMS7-2030
Pathogens / Pathogenicity

BACTERIOCINS AGAINST MYCOBACTERIA: THE CASE OF CIRCULAR PEPTIDE AS-48

C. AGUILAR[1,2,3], B. GRACIA[1,2,3], A. VITORIA[1,2,3], A. LUCIA[1,2,3], R. CEBRIAN[4], M. MAQUEDA[4], J. AINSA[2,3,5]
[1]UNIVERSIDAD DE ZARAGOZA, DEPARTAMENTO DE MICROBIOLOGÍA, ZARAGOZA, Spain
[2]INSTITUTO DE INVESTIGACION SANITARIA ARAGON, IIS-ARAGON, ZARAGOZA, Spain
[3]Instituto de Salud Carlos III, CIBER de Enfermedades Respiratorias CIBERES-, MADRID, Spain
[4]UNIVERSIDAD DE GRANADA, DEPARTAMENTO DE MICROBIOLOGÍA, GRANADA, Spain
[5]UNIVERSIDAD DE ZARAGOZA, DEPARTAMENTO DE MICROBIOLOGÍA- AND BIFI, ZARAGOZA, Spain

Backgrounds

Mycobacteria are highly resistant to antimicrobials, thus leaving very few drugs available for treatment of tuberculosis. Since the incidence of multi-drug resistant strains of *Mycobacterium tuberculosis* is increasing worldwide this is promoting the development of new therapeutic approaches.

Objectives

We have investigated the antimycobacterial activity of peptide AS-48, produced by *E. faecalis*, which is targeting the bacterial membrane, and it is active against several other Gram-positive bacteria.

Methods

Susceptibility was tested by resazurin microtiter assay, using the checkerboard test for determining synergy. Cytotoxicity against cell lines was tested by the MTT and neutral red assays. Changes in membrane functions were tested by DiOC2(3) and ethidium bromide accumulation.

Conclusions

Bacteriocin AS-48 is active against *M. tuberculosis* clinical and reference strains, and against non-tuberculous clinical isolates of other mycobacterial species. The combination of AS-48 with either lysozyme or first line drugs (commonly used in the treatment of tuberculosis) increases bactericidal action of AS-48, showing a synergic interaction. At concentrations close to the MIC of AS-48, we could not detect any cytotoxic effect against THP-1 and MHS macrophage cell lines. In summary, we consider that bacteriocin AS-48 has an interesting potential in the treatment of infectious diseases, including tuberculosis therapy because of its antimicrobial activity and its low cytotoxicity against cell lines.

FEMS7-1308
Pathogens / Pathogenicity

CHARACTERISTICS OF VANCOMYCIN-RESISTANT ENTEROCOCCUS FAECIUM ISOLATES FROM CARIBBEAN COUNTRIES

P.E. Akpaka[1], S. Kissoon[1], P. Jayaratne[2], C. Wilson[3], G.R. Golding[4], A.M. Nicholson[5], D. Lewis[6], S. Hermelijn[7], A. Wilson-Pearson[8], A. Smith[9]
[1]The University of the West Indies, Paraclinical Sciences, St. Augustine, Trinidad & Tobago
[2]McMaster University-, Department of Pathological Sciences, Hamilton-, Canada
[3]King Edward VII Memorial Hospital-, Department of Pathology and Microbiology-, Hamilton, Bermuda
[4]Public Health Agency of Canada, National Microbiology Laboratory, Winnipeg, Canada
[5]The University of the West Indies, Department of Microbiology, Mona Kingston, Jamaica
[6]The University of the West Indies, Faculty of Medical Sciences, Cave Hill, Barbados
[7]Medical Faculty of the Academic University of Suriname, Department of Microbiology, Paramaribo, Surinam
[8]Georgetown Public Hospital Corporation, Microbiology Laboratory, Georgetown, Guyana
[9]King Edward VII Memorial Hospital, Department of Pharmacy, Hamilton, Bermuda

Backgrounds

Global emergence of vancomycin-resistant *Enterococcus faecium* (VRE) has been attributed to the clonal spread of clonal complex 17 (CC17). The CC17 is characterized by resistance to quinolones and ampicillin and presence of enterococci surface protein (*esp*) in majority of isolates.

.

Objectives

This multinational study was undertaken to investigate the antimicrobial resistance patterns, clonal relationships, virulent factors (*esp* and *hyl* genes) and population genetics of VRE isolates recovered from twelve hospitals in eight Caribbean nations

Methods

Seventy VRE isolates recovered from clinical specimens of patients admitted in 12 hospitals in eight Caribbean nations were characterized by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Genes encoding antibiotic resistance and putative virulence traits were investigated by PCR. Antimicrobial susceptibility testing (AST) was conventionally performed and interpreted according to Clinical and Laboratory Standard Institute (CLSI) guidelines. Patient clinical records were analyzed during the isolates collection.

Conclusions

None of VRE isolates from the Caribbean countries is multi-drug resistant and vancomycin resistant is mediated mainly by *van*A gene. Infections associated with VRE isolates in the region were all hospital related. Although all VRE isolates mainly harbored the *esp* gene which is known to be involved in outbreaks, yet there has never been any report of outbreaks of VRE infections in the region. None of the VRE isolates carry *hyl* gene. Predominant sequence types (ST) in the region include ST 412, 750, 203, 736 and 18. All these STs are from the CC17 ancestor, that is similar to clonal complex also circulating in North and South American countries.

FEMS7-0433
Pathogens / Pathogenicity

MICROBIAL COMPOSITION OF PERIIMPLANTITIS ASSOCIATED ORAL BIOFILM AS REVEALED BY CULTURE INDEPENDENT CLONING TECHNIQUE

A. Al-Ahmad[1], F. Muzafferiy[1], A. Anderson[1], J.P. Woelber[1], P. Ratka-Krüger[1], T. Fretwurst[2], K. Nelson[2], E. Hellwig[1]
[1]Albert-Ludwigs-University Freiburg, Operative Dentistry and Periodontology, Freiburg, Germany
[2]Albert-Ludwigs-University Freiburg, Oral- and Craniomaxillofacial Surgery, Freiburg, Germany

Backgrounds

Persistent subgingival oral biofilm associated with oral implants can lead to periimplantitis and subsequently to loss of oral implants. Up to now, it is not clear which pathogens are associated with periimplantitis.

Objectives

In the present clinical study, the microbial composition of the subgingival oral biofilm from oral implants with periimplantitis has been studied in comparison to healthy implants.

Methods

Oral biofilm samples were taken from ten periimplantitis patients who also had at least one healthy oral implant. The biofilm samples of the healthy oral implant sites served as control. After DNA extraction, the 16S rDNA of bacteria and 18S rDNA of fungi were amplified and the PCR products were used to construct clone libraries. The DNA of selected representative clones was sequenced and microbial species were identified by comparing the sequences with public databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The study was approved by the ethics committee of the University of Freiburg.

Conclusions

The results showed an association of the specific periodontal pathogens *Porphyromonas gingivalis, Tannerella forsythia* und *Treponema denticola* with periimplantitis. The proportion of the aforementioned pathogens in the periimplantitis samples was significantly higher than in the subgingival biofilm samples gained from the healthy implants. Furthermore, other microorganisms such as *Peptostreptococcus micra* and *Candida* spp. which are not specific for periodontitis were detected in the biofilm samples from the periimplantitis sites.
Typical periodontal oral pathogens should be considered as associated factors for periimplantitis. The composition of periimplantitis associated biofilm should be considered if antibiotic therapy has to be applied.

FEMS7-2152
Pathogens / Pathogenicity

INCREASED SUSCEPTIBILITY OF AGED C57BL/6 MICE TO LISTERIA MONOCYTOGENES (LM): UPREGULATED CD39/CD73 EXPRESSION IN TREG CELLS AND IMBALANCE OF TH1/TH2 RESPONSES

M.S. Alam[1], C. Cavanaugh[1], K. Williams[1]
[1]US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Laurel, USA

Backgrounds

The case-fatality rate of invasive *Listeria monocytogenes* (*Lm*) infection is >19%. The number of listeriosis cases for elderly is >50%. Aging affects host susceptibility due to an imbalance of protective immunity and T-cell–mediated suppression.

Objectives

We developed a geriatric murine model for listeriosis and investigated *Lm* dose-response and *Lm* susceptibility in the elderly by examining the regulatory role of Th cells.

Methods

Young-adults, middle-aged, and aged C57BL/6 mice were gavaged with incremental doses of *Lm*. Post-infection tissues were examined for bacterial burden and immune response. Splenocytes were analyzed for intracellular Th1/Th2 cytokines (IL-10, IFN-γ), distribution of Treg ($CD4^{+}CD25^{h}$) and Th17 cells before/after infection. The expression of, adenosine (Ado) producing enzymes, CD39 (nucleoside triphosphate dephosphorylase) and CD73 (ecto-5'-nucleotidase) were also analyzed by FACS or qRT-PCR.

Conclusions

Geriatric *Lm*-infected mice lost body-weight dose-dependently, had higher *Lm* colonization and succumbed to infection faster than young-adult infected mice. Uninfected, aged-mice showed a higher baseline pro-inflammatory (IFN-γ) response than young-adult mice. After infection, IFN-γ level was significantly lower in aged-mice as compared to young-adult mice. Increased numbers of Th-17 cells were observed in the aged mice before and after infection. Increased levels of IL-10 and Treg cells were observed in infected aged-mice. Age-dependent upregulated expression of CD39/CD73 was observed in purified Treg cells, suggesting increased Ado (an immune-suppressive-mediator) production. Increased *Lm* susceptibility in aged-mice may be associated with imbalanced anti-inflammatory responses, upregulated expression of CD39/CD73 in Treg-cells and reduced IFN-γ response. Increased *Lm* tissue load in aged-mice seemed unaffected by accumulation of pro-inflammatory splenic Th17 cells.

FEMS7-1892
Pathogens / Pathogenicity

THE IMPAIRED QUORUM SENSING RESPONSE OBSERVED IN ANTIBIOTIC RESISTANT PSEUDOMONAS AERUGINOSA MEXR* MUTANTS IS NOT CAUSED BY THE EXTRUSION OF 3-OXO-C12-HSL THROUGH MEXAB-OPRM EFFLUX SYSTEM

M. Alcalde-Rico[1], *J. Olivares*[2], *J.L. Martínez*[1]
[1]*National Center for Biotechnology- CSIC, Microbial Biotechnology, Madrid, Spain*
[2]*Pontificia Universidad Católica de Chile, Institute of Biology, Valparaiso, Chile*

Backgrounds

Pseudomonas aeruginosa is one important opportunistic pathogen causing many different nosocomial infections difficult to treat due to its low susceptibility to antibiotics. Among the elements involved in resistance, the RND efflux system MexAB-OprM is implied in both intrinsic and acquired resistance. Its overexpression also leads to a lower production of quorum sensing regulated virulence factors, being the extrusion of 3-oxo-C_{12}-HSL by this efflux system the cause proposed for this impaired virulence.

Objectives

Determine the accumulation level of the quorum sensing signal molecules (QSSMs) 3-oxo-C_{12}-HSL, C_4-HSL and PQS/HHQ in both supernatants and cellular extracts from a MexAB-OprM overproducer strain in order to clarify the relevance of each QSSMs in the low QS response observed in *mexR** mutants.

Methods

We analysed in early-stationary phase by real time RT-PCR the expression level of the genes belonging to the Las, Rhl and Pqs regulons controlled by 3-oxo-C_{12}-HSL, C_4-HSL and PQS/HHQ respectively. Different techniques based on biosensor strains to determine the QS signals accumulation were used: i) Thin Layer Chromatography; ii) combined automated luminometer-spectrophotomer assays; iii) chromosomal insertion of *luxCDABE*-based reporter constructions.

Conclusions

The impaired QS response associated to MexAB-OprM overproduction in a *mexR** mutant of *P. aeruginosa* is consequence of the strong defect in C_4-HSL and PQS/HHQ accumulation and not by the slightly affected 3-oxo-C_{12}-HSL production. This issue is opposite to previous claims proposing that which the low virulence phenotype observed in MexAB-OprM overproducer strains would be mainly caused by the extrusion of 3-oxo-C_{12}-HSL through this efflux system.

FEMS7-1396
Pathogens / Pathogenicity

DISSEMINATION OF MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII CLONES AMONG MEXICAN HOSPITALS

M.D. Alcántar-Curiel[1], M.D. Jarillo-Quijada[1], C. Gayosso-Vázquez[1], J.L. Fernández-Vázquez[1], R. Morfín-Otero[2], E. Rodríguez-Noriega[2], J.E. Toledano-Tableros[3], S. Giono-Cerezo[3], M.D.R. López[4], C. Espinosa-Sotero[5], M.A. Morales-Gil[5], J.I. Santos-Preciado[1]
[1]Facultad de Medicina- Universidad Nacional Autónoma de México, Unidad de Investigación en Medicina Experimental, Ciudad de México, Mexico
[2]Hospital Civil de Guadalajara, Enfermedades Infecciosas, Guadalajara- Jalisco, Mexico
[3]Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional, Microbiología, Ciudad de México, Mexico
[4]Hospital Regional "General Ignacio Zaragoza" ISSSTE, Microbiología, Ciudad de México, Mexico
[5]Hospital General de México Dr. Eduardo Liceaga, Pediatría, Ciudad de México, Mexico

Backgrounds

Multi-drug-resistant *A. baumannii* have emerged as a major cause of nosocomial infections in Mexico often serving as reservoirs of resistant determinants. Carbapenem-resistance has been continuously reported among isolates, causing major therapeutic challenges, similarly to what has been described worldwide.

Objectives

The aim of this study was to investigate the hospital dissemination of antimicrobial-resistant *A. baumannii* clones in three Mexican hospitals.

Methods

Two hundred fifty one *A. baumannii* isolates were obtained from three tertiary referral hospitals in two different regions of Mexico: Hospital Civil de Guadalajara, in western Mexico, and Hospital Regional "General Ignacio Zaragoza" ISSSTE and Hospital General de Mexico, in central Mexico from January 2015 to December 2016. Antimicrobial susceptibility was determined by different automatic testing systems. Metallo-beta-lactamases (MBLs) phenotype was confirmed by modified Hodge test and screened for OXA and MBL-encoding genes by PCR. We genotyped and compared the similarities of isolates using *ApaI* digestion and Pulsed Field Gel Electrophoresis (PFGE). Analysis of the PFGE patterns was performed visually to classify the isolates into clones according to the Tenover criteria. Cluster analysis was performed by the unweighted pair group method with mathematical averaging, and DNA relatedness was calculated by using the Dice coefficient.

Conclusions

This study identifies the widespread carbapenem-resistant *A. baumannii* clones involved in nosocomial infections and the inter hospital transmission mainly of one clone in three tertiary referral hospitals from two different geographical regions of Mexico. It also underscores the importance of molecular epidemiology of antimicrobial resistance as an integral part of hospital surveillance.

FEMS7-2773
Pathogens / Pathogenicity

LONGITUDINAL SAMPLING OF ELDERLY ASYMPTOMATIC BACTERURIA PATIENTS HIGHLIGHTS ANTIMICROBIAL RESISTANCE PROPAGATION AND COLONISATION

P. Aldridge[1], L. Drage[1], R. Pickard[2], C. Harding[3], J. Hall[4]
[1]Newcastle University, Centre for Bacterial Cell Biology, Newcastle upon Tyne, United Kingdom
[2]Newcastle University, Institute for Cellular Medicine, Newcastle upon Tyne, United Kingdom
[3]Freeman Hospital, Urology, Newcastle upon Tyne, United Kingdom
[4]Newcastle University, Institute for Cell and Molecular Biosciences, Newcastle upon Tyne, United Kingdom

Backgrounds

Urinary tract infections (UTIs) are the second most common cause of infection worldwide, making them a huge economic burden. People can carry diagnostic loads of bacteria within the bladder without experiencing any symptoms, this is asymptomatic bacteriuria (ABU). ABU can reactivate sporadically, causing symptomatic episodes, known as recurrence. Current treatment is with antibiotics, either short-courses or prophylaxis. Guidelines state to only treat symptomatic episodes. However, UTI symptoms are often diffuse and unclear, especially in older people. Thus, ABU is inappropriately treated in up to 52% of cases, encouraging antibiotic resistance.

Objectives

Better ways of discriminating between symptomatic and asymptomatic cases are needed. Our objective was to analyse potential changes in the host response and the colonising bacteria around ABU to symptomatic transitions, allowing for analysis into potential predictive biomarkers for symptoms.

Methods

We designed a clinical study where 30 patients over 65 years of age with recurrent UTI were recruited for 6 months. Every 2 weeks a urine sample and symptom questionnaire was collected. Urine was analysed for cytokine content and the viable microflora.

Conclusions

Despite varying levels of immune activation, a range of bacterial uropathogenic species were able to evade host-defences and thrive in the bladder long-term. Our study has provided an insight into the long-term colonisation of the ageing bladder and the impact treatment can have on reaching a stabilised microflora. Our study suggests that what the health care system observes and what patients perceive are not necessarily consistent. We argue greater emphasis on medical education could significantly improve patient well-being.

FEMS7-3030
Pathogens / Pathogenicity

PREVALENCE AND MOLECULAR CHARACTERIZATION OF MULTI-DRUG RESISTANT EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING ESCHERICHIA COLI ISOLATED FROM BOVINE MASTITIS

T. Ali[1], S. ur Rahman[2], H. Dandan[1], Z. Limei[1], M. Shahid[1], D. Youtian[1], G. JIan[1], H. Bo[1]
[1]China Agricultural University,
Department of Clinical Veterianry Medicine- College of Veterinary Medicine, Beijing, China
[2]Abdul Wali Khan University, College of Veterinary Sciences and Animal Husbandry, Mardan, Pakistan

Backgrounds

Antimicrobial resistance in bacteria is becoming a serious threat to animals as well as to human health worldwide. Particularly, extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli,* is a major challenge to the modern era.

Objectives

Therefore, this study was conducted to investigate the prevalence of ESBL-producing *E. coli* isolated from bovine mastitis and to characterise these isolates. A total of 1340 mastitic milk samples were collected from different dairy herds located in sixteen provinces of China.

Methods

Screening of ESBL-producing *E. coli* and antibiotic susceptibility was performed according to CLSI guidelines. ESBL encoding genes, multi-locus sequence typing and plasmid typing was performed by PCR and sequence analysis. Among 173 isolated *E. coli*, 49 ESBL-producing *E. coli* were identified, with CXT-M (87.76%; CTX-M-15) being the most prevalent genotype followed by TEM (51.03%) and SHV (13.56%). Two ESBL-positive isolates (CTX-M-15) from bulk milk samples were also identified. All the ESBL-producing isolates were multi-drug resistant, showing high resistance (100% to 55%) to common antibiotics such as beta-lactams, aminoglycoside, tetracycline and fluoroquinolones. Majority of isolates belong to the commensal phylogenetic group A (69.39%), followed by D (20.41%). MLST data showed high molecular diversity among the ESBL-producers. Importantly, majority of ESBL-producing *E. coli* harboured IncF (IncFIA, IncFIB, IncFIC and IncFIIs) plasmids. Furthermore, conjugation experiments exhibited the successful transfer of resistance phenotypes in the transconjugates.

Conclusions

This study concludes on high occurrence of ESBL-producing *E. coli* isolated from dairy cows, which is worrisome for the dairy industry and for the public health as ESBL-genes could rapidly spread via horizontal gene transfer.

reference number: **NSFC No.31572587**

FEMS7-1596
Pathogens / Pathogenicity

VIRUS- AND ANTIBIOTIC- RESISTANCE PHENOTYPES OF NATURAL AND CLINICAL E. COLI

R. Allen[1], K. Pfrunder-Cardozo[1], D. Meinel[2], A. Egli[2], A. Hall[1]
[1]ETH Zürich, Environmental Systems Science, Zürich, Switzerland
[2]University of Basel, Biomedicine, Basel, Switzerland

Backgrounds

Associations among different resistance phenotypes are important for understanding bacterial evolution and designing rational therapies. However we lack a quantitative understanding of how resistance against different stressors and types of stressors are associated in natural and clinical populations.

Objectives

To test this, we measured the resistance phenotypes of 94 isolates of *Escherichia coli* against a wide range of antibiotics and bacteriophages. We then tested for associations among different resistance phenotypes and the predictive power of information such as phylogenetic relatedness and plasmid content.

Methods

We found correlations between some resistance phenotypes across isolates, and this was more common for pairs of stressors of the same type (antibiotic-antibiotic or phage-phage) than different types (antibiotic-phage). Both phage- and antibiotic- resistance were predicted by core genome phylogeny, but only antibiotic resistance was predicted by plasmid content. We then used the correlations among resistance phenotypes to identify genes involved in an uncharacterized phage-resistance mechanism, which we verified using single-gene knockouts and experimental evolution.

Conclusions

This suggests associations among resistance phenotypes offer predictive power over the genetic basis of resistance to uncharacterized or novel antimicrobials. That resistance to antibiotics and phages appears to be evolving independently is encouraging in the context of phage therapy.

FEMS7-1196
Pathogens / Pathogenicity

THE MAPK HOG1 PROMOTES RECOVERY FROM CELL CYCLE ARREST INDUCED BY OXIDATIVE STRESS IN THE PATHOGENIC FUNGUS CANDIDA ALBICANS

R. Alonso-Monge[1], *I. Correia*[2], *E. Román*[1], *D. Prieto*[1], *J. Pla*[1]
[1]*Universidad Complutense de Madrid, Microbiología II, Madrid, Spain*
[2]*IBMC- Instituto para a Investigação e Inovação em Saúde, Instituto de Biologia Molecular e Celular, Porto, Portugal*

Backgrounds

Eukaryotic cell cycle needs to be tightly regulated in order to ensure cell survival. Cell cycle progression depends on environmental conditions while signal transduction pathways play a crucial role in sensing surrounding stresses. In *Saccharomyces cerevisiae* the MAPK (Mitogen Activated Protein Kinase) Hog1 has been implicated in cell cycle arrest induced by osmotic shock. In the opportunistic pathogen *Candida albicans* the HOG pathway has been involved in response to osmotic and oxidative stress. However, there is a lack of knowledge on how cell cycle is regulated in response to stress.

Objectives

1.Analyse the effect of oxidative stress induced by hydrogen peroxide in cell cycle progression in *C.albicans.*
2.Analyse the role of the MAPK Hog1 in cell cycle progression under oxidative stress

Methods

The *C. albicans* wild type strain was tagged with GFP in order to distinguish it from the *hog1* mutant strain. Then, mixed cultures (wild type-GFP /*hog1* mutant strains) were elutriated and G1 phase cells were released in the presence (or not) of hydrogen peroxide. Cell cycle progression was followed in time by Flow Cytometry and cyclins expression was quantified by RT-PCR-Q

Conclusions

1.Hydrogen peroxide induced a transient arrest at G1 phase in *C.albicans* cell cycle.
2.The *hog1* mutant required longer time to resume growth after arrest induced by oxidative challenge
3.The *hog1* mutant progresses faster through the cell cycle under standard growth conditions
3.Expression of certain cyclins (Cln3, Pcl2 and Hgc1) was altered in the *hog1* mutant compare to the wild type strain

FEMS7-1205
Pathogens / Pathogenicity

VIBRIO VULNIFICUS RTXA13 TRIGGERS A CYTOKINE STORM IN MICE

C. Murciano[1], A. Fernández-Bravo[2], C.T. Lee[3], C. Amaro[1]
[1]ERI BioTecMed, Microbiology and Ecology, Burjassot, Spain
[2]ERI BioTecMed, Microbiología y Ecología, Valencia 46100, Spain
[3]College of Medicine- National Cheng-Kung University, Department of Microbiology & Immunology, Tainan, Taiwan R.O.C.

Backgrounds

Vibrio vulnificus biotype 2-serovar E causes death by sepsis in humans/fish. It produces a modular-toxin ($RtxA1_3$) that contains a putative actin-cross-linking domain (ACD). *rtxA1₃* is duplicated in plasmid and chromosome-I. Our hypothesis is that $RtxA1_3$ induces a cytokine-storm at short term that can cause death by sepsis.

Objectives

i) To get single/multiple mutants deficient in ACD/$RtxA1_3$/VvhA (another important cytolysin) and determine its role in cell-death and mouse-virulence. ii) To relate the toxin with cytokine-storm at short term in infected mice. iii) To follow bacterial growth and *rtxA1₃* expression in human blood with and without iron.

Methods

Bacterial mutants: allelic interchange. **Cytotoxicity**: LDH release /flow-cytometry. **Actin-cross-linking activity**: western-blotting. **Virulence**: Lethal-dose-50% after intraperitoneal infection of mice. **Murine immune-response**: by quantifying the transcription of 84 immune-related genes in blood from infected mice by qRT-PCR. **Bacterial invasion in mouse**: Bacterial growth in murine internal organs plus *rtxA1₃*-expression by qRT-PCR**. Bacterial invasion in humans and role of iron**: Bacterial growth in human blood (with or without iron) plus *rtxA1₃* expression by qRT-PCR

Conclusions

i) $RtxA1_3$ causes death by necrosis/apoptosis in human endotheliocytes/monocytes; ii) ACD has actin-cross-linking activity; iii) Biotype 2-serovar E is only successful in invasion in iron-overloaded humans; iv) $RtxA1_3$ induces the up-regulation of 47 murine genes at 4h post-infection: some involved in immune-response against intracellular-pathogens (interferon-related, *Tlr9* and *Nod2*…) and pyroptosis (*Pycard*, *Casp1* and *Nlrc4*). The crucial role of $RtxA1_3$ in inducing a cytokine-storm is ground-breaking, as it is the first description of a bacterial toxin, apart from superantigens, involved in this life-threatening immune-response.

FEMS7-1548
Pathogens / Pathogenicity

IN VITRO ACTIVITIES OF SIX ANTIFUNGAL DRUGS AGAINST CANDIDA GLABRATA ISOLATES: AN EMERGING PATHOGEN

N. Amirrajab[1], N. Amirrajab[1], T. Shokohi[2], H. Badali[2], M. Didehdar[3], M.H. Afsarian[4], R. Mohammadi[5], N. Lotfi[2]
[1]Ahvaz Jundishapur Univeristy of Medical Scineces - Ahvaz - IRAN, Laboratory Sciences, ahvaz, Iran
[2]Mazandaran University of Medical Sciences- Sari- Iran,
Department of Medical Parasitology and Mycology, Sari, Iran
[3]Arak University of Medical Sciences- Arak- Iran, Department of Medical Mycology and Parasitology-, Arak, Iran
[4]Fasa University of Medical Sciences- Fasa- Iran, Deptment of Microbiology, Fasa, Iran
[5]Isfahan University of Medical Sciences- Isfahan- IR Iran,
Department of Medical Parasitology and Mycology, Isfahan, Iran

Backgrounds

Candida glabrata is a pathogenic yeast with several unique biological features and associated with an increased incidence

rate of candidiasis. It exhibits a great degree of variation in its pathogenicity and antifungal susceptibility.

Objectives

The aim of the present study was to evaluate the in vitro antifungal susceptibilities of the following six antifungal drugs

against clinical *C. glabrata* strains: amphotericin B (AmB), ketoconazole (KTZ), fluconazole (FCZ), itraconazole (ITZ), voriconazole

(VCZ), and caspofungin (CASP).**Methods**

Forty clinical *C. glabrata* strains were identifed using DNA sequencing. The in vitro antifungal susceptibility

was determined as described in clinical laboratory standard institute (CLSI) documents (M27-A3 and M27-S4).**Conclusions**

These findings confirm that CASP, compared to the other antifungals, is the potent agent for treating candidiasis

caused by *C. glabrata*. However, the clinical efficacy of these novel antifungals yet to be determined.

FEMS7-1956
Pathogens / Pathogenicity

SEROPREVALENCE OF TOXOPLASMA GONDII IN THE IRANIAN GENERAL POPULATION: A SYSTEMATIC REVIEW AND META-ANALYSIS

A. Ahmad Daryani[1], M. Mehdi Sharif[1], S. Shahabeddin Sarvi[1], A. Azadeh Mizani[1], A. Afsaneh Amouei[1]
[1]Toxoplasmosis Research Center- Mazandaran University of Medical Sciences- Sari- Mazandaran-4847191971- Iran, Parasitology and Mycology, Sari, Iran

Backgrounds

Toxoplasma gondii is one of the most common protozoan parasites with widespread distribution globally. While *T. gondii* infection in healthy people is usually asymptomatic, it can lead to serious pathological effects in congenital cases and immunodeficient patients.

Objectives

We sought to identify the seroprevalence rate of *Toxoplasma* infection in the Iranian general population to develop a comprehensive description of the disease condition in Iran for future use.

Methods

8 Electronic databases and Persian language databases were searched. Furthermore, graduate student dissertations and proceedings of national parasitology congresses were searched manually.

Conclusions

Our search resulted in a total of 35 reports published from 1978 to 2012.These include 52,294 individuals and 23,385 IgG seropositive cases. The random errors method was used for this meta-analysis. The result shows that the overall seroprevalence rate of toxoplasmosis among the general population in Iran was 39.3% (95% CI = 33.0%–45.7%). There was no significant difference in the seroprevalence rate between male and female patients. In addition, the data indicates that there are high seroprevalence in groups who have direct contact with cats, consume uncooked meat and raw fruits or vegetables, in farmers and Housewife, individuals who have a low level of education, and live in rural areas. To the best of our knowledge, this is the first systematic review of *T. gondii* infection seroprevalence in Iran, which shows a high prevalence of *Toxoplasma* infection (more than one third). We highly recommend further study for the purposes of aiding patient management and developing more efficient diagnostic tests and effective prevention approaches.

FEMS7-2274
Pathogens / Pathogenicity

SCOUTING NEW ANTIMICROBIALS: THE FAD SYNTHETASE AS A NOVEL PROMISING TARGET

E. Anoz-Carbonell[1,2,3], A. Lucía Quintana[2], M. Sebastián[1,3], S. Salillas[1,3], J. Sancho[1,3], J.A. Aínsa[1,2], M. Medina[1,3]
[1]Instituto de Biocomputación y Física de Sistemas Complejos BIFI- Universidad de Zaragoza, Instituto de Biocomputación y Física de Sistemas Complejos BIFI- Universidad de Zaragoza, Zaragoza, Spain
[2]Dpto. Microbiología- Medicina Preventiva y Salud Pública- Universidad de Zaragoza and CIBER Enf ermedades Respiratorias CIBERES- Instituto de Salud Carlos III, Dpto. Microbiología- Medicina Preventiva y Salud Pública- Universidad de Zaragoza and CIBER Enfe rmedades Respiratorias CIBERES- Instituto de Salud Carlos III, Zaragoza, Spain
[3]Departamento de Bioquímica y Biología Molecular y Celular- Universidad de Zaragoza, Departamento de Bioquímica y Biología Molecular y Celular- Universidad de Zaragoza, Zaragoza, Spain

Backgrounds

The prokaryotic FAD synthetases (FADS) are bifunctional enzymes that catalyze the biosynthesis of the essential cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FADS are key proteins in flavin and flavoproteome homeostasis, since the alteration of their enzymatic activities leads into a FMN and FAD deficiency, and consequently into the accumulation of the apo forms of numerous flavoproteins, which are unable to carry out their expected functions in the cellular metabolism and other essential processes.

Objectives

This crucial role in cellular metabolism, together with the significant differences with their eukaryotic counterparts, converts the FADS in an attractive potential drug target for the development of inhibitors endowed with antimicrobial activity.

Methods

Through the high-throughput screening based on the inhibition of CaFADS and SpnFADS (FADS from *Corynebacterium ammoniagenes* and *Streptococcus pneumoniae,* representative members of the prokaryotic FADS), we have identified 40 potential inhibitors. These compounds have been thoroughly characterized by the determination of their antimicrobial spectrum and their cytotoxic effect on eukaryotic cells.

Conclusions

Only 5 compounds show antimicrobial activity against Gram-positives, and one of them against Gram-negative microorganisms as well. Nevertheless, their therapeutic application could be limited due to their cytotoxicity on eukaryotic cells at the concentrations required for the antimicrobial activity. Both antibacterial and cytotoxic effects may be associated with the unspecific alteration of other essential flavoproteins and flavoenzymes.

In a further step, the molecular information of the antimicrobial mechanism will be used for the optimization of these compounds in order to generate second-generation antimicrobials with higher efficacy and less toxicity.

FEMS7-1374
Pathogens / Pathogenicity

IMPAIRED ANTI-FIBROTIC EFFECT OF BONE MARROW-DERIVED MESENCHYMAL STEM CELL IN A MOUSE MODEL OF PULMONARY PARACOCCIDIOIDOMYCOSIS

J. Arango[1,2], J.D. Puerta-Arias[2], P.A. Pino-Tamayo[3], L.M. Salazar-Peláez[4], A. González[1,5]
[1]Universidad de Antioquia, Escuela de Microbiolgía, Medellín, Colombia
[2]Universidad de Antioquia, Medical and Experimental Mycology Group- CIB, Medellín, Colombia
[3]Weill Cornell Medical College, Department of Microbiology and Immunology, New York, USA
[4]Universidad CES, Basic Sciences Group, Medellín, Colombia
[5]Universidad de Antioquia, Basic and Applied Microbiology Research Group MICROBRA, Medellín, Colombia

Backgrounds

Paracoccidioides spp is the causal agent of paracoccidioidomycosis (PCM), an endemic and systemic mycosis widely distributed in Latin America; this disease is characterized by a chronic granulomatous inflammatory response with development of pulmonary fibrosis (PF), even after antifungal treatment. Recently, it has been demonstrated that bone marrow-derived mesenchymal stem cells (BM-MSCs) therapy could be used as an alternative to treat chemical-induced PF.

Objectives

To evaluate the anti-fibrotic therapeutic potential effect of BM-MSCs in a mouse model of pulmonary PCM.

Methods

BM-MSCs were isolated and purified from BALB/c mice using standardized methods. BALB/c male mice were inoculated i.n. with $1.5x10^6$ *P. brasiliensis* yeasts. An additional group of mice was treated with itraconazole (ITC) at the 6^{th} week p.i.; $1x10^6$ BM-MSCs were administered i.v. at eight weeks p.i. to all groups of mice in a single dose; animals were sacrificed on the 12th week p.i. in order to evaluate: fibrocytes counts, soluble collagen, pro-fibrotic genes expression and histopathological aspects.

Conclusions

BM-MSCs-treated mice showed a significant increase of fibrocytes, soluble collagen and collagen-3a1 expression in comparison with infected untreated-mice. Lung histopathological analysis showed an increased of granulomas and collagen deposition in those BM-MSCs treated-mice. Interestingly, the combined therapy BM-MSCs/ITC induced a reduction of TIMP-1 gene expression in comparison with the respective control groups. This is the first study to evaluate the effect of BM-MSCs therapy in PCM; however, these results indicate that the immunoregulatory function of BMSCs, maybe triggered by the interaction with *P. brasiliensis*, exacerbates the pulmonary fibrosis response.Supported: Colciencias (project_358-2011)

FEMS7-3103
Pathogens / Pathogenicity

ROLE OF C. DIFFICILE C-DI-GMP REGULATED PROTEINS IN BIOFILM FORMATION AND ADHESION TO HUMAN CELLS

V. Arato[1], *R. Leuzzi*[1]
[1]*GSK Vaccines, AIMB, Siena, Italy*

Backgrounds

C. difficile (*CD*) is a Gram-positive and anaerobic bacterium which causes several gut diseases. Like other bacteria, *CD* modulates the transition from a motile to a sessile lifestyle through a mechanism of riboswitch regulated by the second messenger cyclic diguanosine monophosphate (c-di-GMP).

High levels of c-di-GMP are involved in the regulation of *CD* colonization processes by modulating the expression of an extracellular protease and surface proteins. The zinc metalloprotease CD2830 has been shown to cleave the putative adhesin CD2831 likely preventing its contribution to adhesion to the host. Importantly, both genes are located downstream of a c-di-GMP riboswitch and intracellular c-di-GMP concentrations modulate adhesive phenotypes by alternatively expressing CD2831 or CD2830.

Objectives

Our studies aim to understand the role of c-di-GMP signaling in the lifecycle of *CD* and its effect on the above-mentioned proteins CD2830 and CD2831.

Methods

To determine the role of c-di-GMP in *CD*, we constitutively expressed the *dccA* gene encoding adiguanylate cyclase enzyme, which synthesizes c-di-GMP. This, together with knocking-out CD2830 and CD2831 genes will contribute to understand the role of the two proteins in biofilm formation *in vitro*. Moreover, in order to study the binding of *CD* to human cells we implemented a 3D co-culture of fibroblasts, enterocytes and mucus-secreting cells representing the human intestinal epithelium.

Conclusions

Preliminary results show that high levels of c-di-GMP promote the down-regulation of CD2830 and therefore the expression of CD2831 on the cell wall. This condition shows to facilitate biofilm formation *in vitro*.

FEMS7-2844
Pathogens / Pathogenicity

MANIPULATION OF THE NADH/NAD+ RATIO TO DISCLOSE THE MECHANISMS UNDERLYING BACTERICIDAL ACTIVITY OF ANTIBIOTICS IN PSEUDOMONAS AERUGINOSA

A. Arce-Rodriguez[1], *S. Häußler*[1]
[1]*Helmholtz Centre for Infection Research, Molecular Bacteriology, Braunschweig, Germany*

Backgrounds

The metabolic plasticity encoded in the genome of *Pseudomonas aeruginosa*, endow this pathogen with the genetic arsenal required to surpass antimicrobial treatments. However, the mode of killing of antimicrobials and the mechanisms underlying its resistance in this bacterium are not fully understood. Recent theories suggest a common mechanism of antimicrobial killing by alterations in the metabolism, inducing the hyperoxidation of NADH in the respiratory chain and thus leading to the formation of reactive oxygen species.

Objectives

In this work we seek to manipulate the intracellular concentrations of NADH/NAD^+ to study this effect in the context of antibiotic killing and antibiotic resistance in *P. aeruginosa*.

Methods

The coding sequence of the NADH oxidase from *Streptococcus pneumoniae* (Nox^{Sp}) and the NADH-producing formate dehydrogenase from *Candida boidinii* ($FDH1^{Cb}$) were overexpressed into *P. aeruginosa* PA14. Both enzymes were shown to be fully functional, decreasing (Nox^{Sp}) or increasing ($FDH1^{Cb}$) the intracellular NADH/NAD^+ ratios.

Conclusions

Transcriptional profile by RNA-Seq of both overexpressing strains showed pleiotropic effects. The oxidation of NADH by Nox^{Sp} induced the overexpression of general stress factors (mainly related to energy unbalance due to the waste of NADH), chemotaxis and virulence (*las*AB, *rhl*, *rpo*S, alkaline proteases and phenazines). Moreover, ribosomal proteins, tRNA metabolism, transporters and the H^+-transporting ATPase were downregulated. The induction of $FDH1^{Cb}$ caused opposite effects, down-regulating genes related to chemotaxis and virulence, and up-regulating ribosomal proteins, tRNA metabolism and genes involved in antibiotic resistance like multidrug-efflux pumps and β-lactamases. Interestingly, $FDH1^{Cb}$-overexpressing cells resisted higher concentrations of ciprofloxacin when compared to empty plasmid-bearing cells.

FEMS7-0337
Pathogens / Pathogenicity

OXIDATIVE STRESS IS AN IMPORTANT COMPONENT IN THE FUNGICIDAL ACTION EXERTED BY POLYENES AND ECHINOCANDINS AGAINST CANDIDA ALBICANS

J.P. Guirao-Abad[1], R. Sánchez-Fresneda[1], B. Alburquerque[1], E.I. Agüera[1], J.C. Argüelles[1]
[1]Universidad de Murcia, Genética y Microbiología, MURCIA, Spain

Backgrounds

Our knowledge on the action mechanisms of the main types of antifungals used in medical practice is still incomplete. A convenient strategy to prevent the appearance of resistance would involve the elucidation of whole sets of fungicidal activities, which, for some established compounds, is currently far from being reached

Objectives

To elucidate the role played by the intracellular formation of reactive oxygen species (ROS) in the fungicidal action carried out by Amphotericin B (AmB) and Micafungin (MF) against Candida albicans

Methods

Clinical MICs were determined using the CLSI protocol and cell survival by viable counting. The oxidative burst was assessed by flow cytometry (dihydrofluorescein diacetate) and the antioxidant enzymatic activities (Catalase, Superoxide dismutase and Glutathione reductase) by spectrophotometric quantification. Intracellular trehalose was measured with purified trehalase following the glucose-oxidase method. Any damage to cell integrity and the structural alterations caused by both antifungals were recorded by optical and electronic microscopy.

Conclusions

The clinical MICs for MF and AmB were 0.016 and 0.012 mg/L, respectively. AmB (0.5-1.0 x MIC) induced a high degree of cell killing accompanied by significant ROS production in exponential Candida albicans SC5314 cell, while the fungicidal effect of MF led to a low level of intracellular ROS formation. Preincubation with thiourea suppressed ROS generation with a concomitant increase in cell viability, regardless of the antifungal applied. The simultaneous measurement of several antioxidant enzymes revealed strong AmB-induced activation of enzymatic activities, whereas MF had only a weak stimulating effect. Likewise, AmB but not MF promoted a conspicuous endogenous synthesis of trehalose, which is a specific protector against oxidative stress in C. albicans.
Our results allow to conclude that the induction of internal oxidative stress in C. albicans through the accumulation of ROS is an important component of the antifungal action of polyenes (AmB) but not of echinocandins (MF).

FEMS7-0980
Pathogens / Pathogenicity

SENSITIVITY OF CANDIDA ALBICANS TREHALOSE-DEFICIENT MUTANTS TO AMPHOTERICIN B AND MICAFUNGIN

R. Sánchez-Fresneda[1], J.P. Guirao-Abad[1], V. Pujante[1], B. Alburquerque[1], J.C. Arguelles[1]
[1]Universidad de Murcia, Genética y Microbiología, MURCIA, Spain

Backgrounds

Trehalose has been proposed as a potential target for new antifungals.

Objectives

The putative role of trehalose as cell protector against antifungal exposure with AmB and MF has been investigated in the null mutants *tps1/tps1* and *tps2/tps2*, which are deficient in trehalose synthase and trehalose phosphatase activity, respectively in *Candida albicans*.

Methods

Cultures were grown in YPD until exponential phase and then treated with both antifungal at 37ºC for 1 h. Viability was determined in samples diluted with sterile water by plating in triplicate on solid YPD after incubation for 2 days at 37ºC. The antioxidant enzymatic activities (Catalase, Superoxide dismutase and Glutathione reductase) by spectrophotometric quantification. Intracellular trehalose was measured with purified trehalase following the glucose-oxidase method.

Conclusions

The two homozygous mutants (*tps1Δ* and *tps2Δ*) showed different cell sensitivity upon exposure with the antifungals AmB (0.25 µg/ml) and MF (0.05 µg/ml). Whereas *tps1Δ* cells were very susceptible to AmB, they showed high resistance to MF. Conversely, *tps2Δ* displayed an opposite degree of sensitivity. Catalase activity was inhibited by both antifungals in the two mutants. Surprisingly, however, MF induced a conspicuous SOD activation only in *tps1Δ*. The opposite pattern in SOD activity was recorded upon AmB exposure. As regards trehalose synthesis, only AmB triggered a marked accumulation of the disaccharide, while MF had a negligible effect.

Collectively, our results suggest that the enzymes involved in trehalose biosynthesis display a different pattern of sensitivity to AmB and MF.

FEMS7-1699
Pathogens / Pathogenicity

GENOME SEQUENCING OF TREPONEMA PALLIDUM REVEALS A PANDEMIC CLUSTER AND PROVIDES INSIGHTS INTO ITS ORIGINS

N. Arora[1]
[1]*University of Zurich, IEU/IRM, Zurich, Switzerland*

Backgrounds

Syphilis, a multi-stage infection caused by the spiral shaped bacterium *Treponema pallidum pallidum (TPA)*, has been re-emerging globally in recent decades despite the availability of effective treatment with antibiotics. Little is known about the genetic patterns of current infections or the evolutionary origins of the disease due to the challenges in obtaining DNA from clinical samples. Moreover, TPA is non-cultivable and displays limited genetic diversity. While resistance to penicillin has not been identified, there has been an increase in the number of strains that do not respond to treatment with the second line antibiotic azithromycin.

Objectives

Our aims are to examine the patterns of genetic diversity and differentiation, especially focusing on samples from contemporary infections, and to gain insights into the history of TPA.

Methods

We used DNA capture and whole genome sequencing to obtain genome-wide data from syphilis patient specimens, combining it with laboratory samples of TPA and two other subspecies (*T. pallidum* subsp. *pertenue*, TPE, and *T. pallidum* subsp. *endemicum*, TEN).

Conclusions

Our phylogenetic analyses using genome-wide sequences point to a common ancestor for all TPA strains in the 18th century. Our analyses also show that most strains from contemporary infections have the mutation associated with azithromycin resistance. Furthermore, most of the clinical strains form part of a globally dominant cluster, named SS14-Ω, which diversified after the discovery of antibiotics in the mid-20th century. The recent phylogenetic expansion of this cluster and its wide geographical distribution are indicative of the emergence and expansion of a pandemic strain.

FEMS7-1298
Pathogens / Pathogenicity

TN1721, A VECTOR OF CHOICE FOR ANTIBIOTIC RESISTANCE IN AEROMONAS SALMONICIDA?

S.A. Attere[1,2,3], *A.T. Vincent*[1,2,3], *M. Paccaud*[1,2,3], *S.J. Charette*[1,2,3]
[1]*Université Laval, Microbiology, Quebec, Canada*
[2]*Université Laval, Institut de biologie intégrative et des systèmes, Quebec, Canada*
[3]*Université Laval, Institut Universitaire de cardiologie et de pneumologie de Québec, Quebec, Canada*

Backgrounds

Antibiotic resistance is a major worldwide problem for fish farming since many waterborne pathogenic bacteria harbor a large panel of drug resistance. Resistance genes can be often acquired through mobile genetic elements such as non-composite transposons. This is the case for *Aeromonas salmonicida* subsp. *salmonicida,* a bacterium causing furunculosis and having a large repertoire of antibiotic resistance genes introduced by mobile elements. Tn*1721*, a non-composite transposon discovered in this microorganism, is known to be a vector for the dissemination of the tetracycline resistance.

Objectives

This study investigates how the spread of Tn*1721* took place in *Aeromonas salmonicida* subsp. *salmonicida*, in correlation with the plasmidome.

Methods

Plasmid profiling was performed by PCR genotyping and also by plasmid extraction, followed by an enzymatic digestion and a migration on an electrophoresis gel. Plasmidic DNA of interest was then sequenced using Illumina technology. Finally, a bioinformatic characterization of the new plasmids was performed to determine their features, including antibiotic resistance genes and transposons.

Conclusions

Tn*1721* was found in two new plasmids of *A. salmonicida* subsp. *salmonicida*. In the large plasmid pAsa8 (110,6 kb), it is fully present but interrupted by two other mobile genetic elements. However, in the small plasmid pAsa10 (10 kb), only the part providing the tetracycline resistance is present. Also, the insertion of the transposon probably occurred through a different mechanism in the case of pAsa10 according to the orientation of the inverted repeats. This study sheds light on an unsuspected importance and diversity of the Tn*1721* in *A. salmonicida* subsp. *salmonicida*.

FEMS7-0689
Pathogens / Pathogenicity

DUAL REPRESSION OF A BACTERIAL PROMOTER BY THE HISTONE-LIKE NUCLEOID STRUCTURING PROTEIN (H-NS) AND THE LYSR-TYPE TRANSCRIPTION FACTOR LEUO

J. Ayala Figueredo[1], *J. Benitez*[1], *A. Silva*[1]
[1]*Morehouse School Of Medicine, Biochemistry Microbiology And Immunology, Atlanta, Usa*

Backgrounds

The *vieSAB* operon encodes a three-component system that regulates virulence gene expression in *V. cholerae* O1, the causative agent of cholera. Transcription of *vieSAB* is silenced by the nucleoid-associated protein H-NS.

Objectives

The objective of this study was to identify antagonists of H-NS repression.

Methods

An assay for proteins that can bind to the *vieSAB* promoter identified the stationary phase regulator LeuO. H-NS and LeuO interacted with overlapping sequences at the *vieSAB* promoter with Kd values of 44.1 and 31.2 nM, respectively. Furthermore, LeuO displaced pre-bound H-NS from the promoter. However, ectopic expression of LeuO from the *araBAD* promoter indicated that LeuO functions as a transcriptional repressor at this locus. To examine the cooperation between H-NS and LeuO in the silencing of *vieSAB* expression, we conducted *in vitro* transcription at two concentrations of RNA polymerase holoenzyme (Es^{70}) (25 and 50 nM). LeuO inhibited *vieSAB* transcription with similar IC_{50} at both Es^{70} concentrations. In contrast, H-NS inhibited *vieSAB* transcription with IC_{50} values of 3.65 and 0.83 nM at low and high Es^{70} concentration, respectively.

Conclusions

We propose a model in which H-NS silences *vieSAB* transcription during exponential growth when the concentration of Es^{70} is highest. In the stationary phase, the concentration of Es^{70} decreases due to sigma factor competition and H-NS exerts weaker repression. At this stage, LeuO is expressed to assist in the silencing of *vieSAB* transcription. This is a rare example in which two repressors target similar recognition sequences at a promoter to effectively silence gene expression at different physiological stages.

FEMS7-0372
Pathogens / Pathogenicity

GENETIC CHARACTERIZATION OF THE VIRULENCE PROPERTIES AND PATHOGENICITY OF BORDETELLA BRONCHISEPTICA STRAIN HT200 ISOLATED FROM A THERMAL SPRING

J. Badhai[1], S.K. Das[1]
[1]Institute of Life Sciences, Division of Gene Function and Regulation, Bhubaneswar, India

Backgrounds

The classical *Bordetella* species *B. pertussis*, *B. parapertussis,* and *B. bronchiseptica*, are obligate parasites of mammalian hosts including humans which exclusively infect the respiratory epithelium causing a range of severe, often fatal, respiratory diseases. Although *B. bronchiseptica* has been found to survive in natural environments such as lake water, so far there is no report on any environmental isolate. Strains of these classical species are very closely related at the genetic level and divide into complexes I-IV, yet are distinct pathogens in terms of virulence, disease severity, persistence, and host specificity. Phylogenetic analysis based on 16S rRNA gene revealed the strain HT200, isolated from warm spring water with temperature 40 °C, belonged to the classical Bordetellae.

Objectives

To characterize the virulence and pathogenicity of *Bordetella bronchiseptica* strain HT200 isolated from warm spring water.

Methods

Methods used includes PCR based genotyping, serum agglutination and immunoblotting assays for detection of virulence factors, mice trachea and lungs colonization, and antibiotic susceptibility test.

Conclusions

Sequence analysis of genes encoding various housekeeping functions, the common *Bordetella* virulence factors including adhesins, autotransporters, and the virulence regulatory system suggests strain HT200 to have evolved divergently from a distinct lineage of *Bordetella bronchiseptica*-like ancestor. It does not produce some of the major virulence factors including pertussis toxin, adenylate cyclase toxin and dermonecrotic toxin produced by clinical isolates of the classical species. However, it colonizes the trachea and lungs in mice. It is resistant to multiple antibiotics. Genotypic and functional divergence compared to the host-restricted pathogenic classical Bordetellae indicates its niche adaptions.

FEMS7-3026
Pathogens / Pathogenicity

IDENTIFICATION OF THE SIDEROPHORE PISCIBACTIN AS A RELEVANT VIRULENCE FACTOR FOR VIBRIO ANGUILLARUM SEROTYPE O2

M. Balado Dacosta[1], J.C. Fuentes-Monteverde[2], J.M. Cabaleiro[1], J. Rodríguez[2], C. Jiménez[2], M.L. Lemos[1]
[1]Institute of Aquaculture, Department of Microbiology and Parasitology, Santiago de Compostela, Spain
[2]Facultad de Ciencias, Departamento de Química Fundamental, A Coruña, Spain

Backgrounds

Vibrio anguillarum causes vibriosis, an haemorrhagic septicaemia that affects many cultured marine fish species worldwide. Siderophores are key virulence factors since they participate in iron uptake from host sources during bacterial infections. In *V. anguillarum* two catechol siderophores, vanchrobactin and anguibactin, were previously identified. While vanchrobactin is a chromosomally encoded system widespread in all pathogenic and environmental strains, anguibactin is a plasmid encoded system restricted to serotype O1 pathogenic strains. An analysis of the genome sequence of strain RV22 (serotype O2) shows that it contains a gene cluster that could encode a siderophore related to piscibactin, the siderophore of *Photobacterium damselae* subsp *piscicida*.

Objectives

The main goal of the present work was to identify this third siderophore in *V. anguillarum,* analyze its role in the cell fitness and determine its contribution to virulence.

Methods

In silico analysis allowed the identification of a genomic island that encodes the piscibactin-like siderophore and the prediction of its chemical structure. The characterization of the siderophore by LC-HRMS confirmed that its stucture corresponded to piscibactin.

Conclusions

The construction of single and double biosynthesis mutants for vanchrobactin and/or piscibactin allowed us to study its contribution to iron uptake and its role in virulence. The results showed that *V. anguillarum* serotype O2-O10 isolates harbour a genomic island that codes enzymes for synthesis of piscibactin. Interestingly, vanchrobactin and piscibactin are simultaneously produced, but piscibactin contribute more than vanchrobactin to virulence for turbot. It is noteworthy that the genomic island encoding piscibactin is widespread in many other *Vibrio* species.

FEMS7-0603
Pathogens / Pathogenicity

UNCOVERING THE ROLE OF ETHYLENE PRODUCED BY PENICILLIUM DIGITATUM IN THE PATHOGENICITY TOWARDS CITRUS FRUIT

A.R. Ballester[1], *L. González-Candelas*[1]
[1]*Instituto de Agroquímica y Tecnología de Alimentos IATA-CSIC, Food Biotechnology, Paterna, Spain*

Backgrounds

Infection of citrus fruit by *Penicillium digitatum* is accompanied by a large increase in ethylene production. Classical studies have shown that this phytohormone is produced both by the fruit and the pathogen. There are three known routes for ethylene production. In plants, ethylene is produced in a two-step reaction from methionine via S-adenosyl-methionine (SAM), which is converted into aminocyclopropane-1-carbxoxylate (ACC) by AAC synthase. Then, ACC oxidase converts ACC into ethylene and cyanide. Many microorganisms also produce ethylene, but using two different pathways. In one of them, methionine is also the precursor compound, but in this pathway it is transaminated into 2-keto-4-methylthiobutyric acid (KMBA), which is spontaneously oxidized to produce ethylene. The last known pathway for ethylene production utilizes α-ketoglutarate (AKG), which derives from glutamic acid, and arginine as substrates in a reaction catalyzed by an ethylene-forming enzyme (EFE), an enzyme that requires ferrous iron and oxygen, yielding ethylene, carbon dioxide, succinate, 1-pyrroline-5-carboxylic acid and guanidine. These last two pathways have been shown to be present in *P. digitatum*. Under *in vitro* growth conditions, the KMBA pathway is the predominant in shake cultures grown in liquid medium, whereas the AKG pathway is predominant in static cultures.

Objectives

To elucidate the contribution of EFE to ethylene production during infection of citrus fruit by *P. digitatum*.

Methods

We have obtained a *P. digitatum* Δ*efeA* knockout mutant using *Agrobacterium*-mediated transformation.

Conclusions

We will present the characterization of this mutant as a valuable tool to understand the role of ethylene in the virulence of *P. digitatum*.

FEMS7-2916
Pathogens / Pathogenicity

EPIDEMIOLOGY OF MULTI-RESISTANT BACTERIA IN THE HOSPITAL ENVIRONMENT OF HIGH-RISK INFECTIOUS UNITS, IBN TOFAIL HOSPITAL- UNIVERSTARY HOSPITALCENTER OF MARRAKECH

A. El Mekes[1, 2], *K. Zahlane*[1], *L. Ait Said*[1], *A. Tadlaoui Ouafi*[3], *M. Barakate*[1]
[1]*Laboratoire des analyses médicales, Hôpital Ibn Tofail, CHU Marrakech*
[2]*Laboratoire de Biologie et Biotechnologie des Microorganismes, Faculté des Sciences Semlalia, Université Cadi Ayyad-Marrakech*
[3]*Laboratoire de Biotechnologie et Bio-ingenierie moléculaire, Faculté des sciences et techniques, Université Cadi Ayyad-Marrakech*

Abstract:

The objective of this study is to analyze the qualitative and quantitative microbial composition of the environment of high risk infectious units at the Ibn Tofail hospital, CHU Mohammed VI, Marrakech.

Material and Methods: This is a prospective study carried out at four units (two operating units, two adult intensive care units Intensive Cure Unit (ICU) of CHU Mohammed VI Marrakech during a period of four months. The samples concerned inanimate surfaces and the hands of different staff. The level of antibiotic resistance was studied by the diffusion method agar medium. The choice of antibiotics and the criteria for interpretation of the antibiogram were made according to the standards of the European Committee on Antibiograms (EUCAST).

Results: 95 bacterial strains were isolated from the 125 samples. The antibiotic resistance profile showed that 46% were multidrug resistant strain, 19% of them were *acinetobacter baumannii* resistant to imipenem (ABRI), 17 % of the *enterobacteriacae* producing extended spectrum of beta-lactamase (ESBLE), and 8 % were methicillin-resistant *Stapylococcus aureus* (MRSA). The lowest rate (4%) was obtained for *Pseudomonas aeruginosa* resistant to carbapenem (PARC). The ABRI was mainly found in the inanimate surfaces of ICU, the EBLSE were predominant in the surfaces of the operating units. However, the MRSA was isolated mostly from the staff handprints and the surfaces of the four studied units.

Conclusion: The alarming presence of MDR bacteria in the hospital environment urges the hospital actors (biologists, hygienists, clinicians, nursing staff) to double their efforts to control these bacteria.

FEMS7-2412
Pathogens / Pathogenicity

NOVEL ANTIINFLAMMATORY, ANTIMICROBIAL AND ANTIBIOFILM PEPTIDE

S. Bárcena-Varela[1], G. Martínez de Tejada[1], L. Heinbockel[2], K. Brandenburg[2]
[1]Universidad de Navarra, Department of Microbiology, Pamplona, Spain
[2]Research Center Borstel, Clinical & Experimental Pathology, Borstel, Germany

Backgrounds

Antimicrobial peptides (AMPs) produced by the innate immune system are promising agents for antibiotic development because these molecules bind to essential and highly conserved structural targets on the bacterial surface. Our group developed a new class of AMPs and demonstrated that the leading candidate, Aspidasept® I (Pep19-2.5), protects mice and rabbits against sepsis caused by an otherwise lethal bacterial infection. However, due to its modest antibacterial activity Aspidasept® I has to be co-administered with antibiotics to be effective. Now, we have synthesized another peptide, Pep19-4LF Aspidasept® II (Pep19-4LF), displaying a much better profile of antimicrobial activity.

Objectives

I, to characterize the antimicrobial, antiinflammatory and antibiotic-enhancing activity of Pep19-4LF. II, to evaluate its capacity to prevent the formation and/or to eliminate bacterial biofilms.

Methods

Antimicrobial activity and antibiotic-enhancing activity was measured using conventional MIC/MBC testing and growth kinetics in the Bioscreen system, respectively. A mouse model of endotoxemia was used to characterize the peptide's ability to neutralize sepsis. The anti-biofilm activity was measured on *P. aeruginosa* biofilms grown in the CDC Biofilm Reactor or using peptide immobilized on the surface of silicone.

Conclusions

Pep19-4LF exhibited a broad spectrum of antimicrobial activity against Gram-positive and Gram-negative strains with MICs as low as 2 µg/mL and acted in synergy with several classes of antibiotics against multi-resistant Gram-negative pathogens.. The anti-biofilm activity of the peptide was comparable to that of levofloxacin. When immobilized, Pep19-4LF prevented the formation of *P. aeruginosa* biofilm and killed preformed biofilm of that organism as efficiently as a colistin coating.

FEMS7-3233
Pathogens / Pathogenicity

FIRST REPORT OF MASS MORTALITIES IN NATURAL POPULATION OF PINNA NOBILIS. A MICROBIAL PERSPECTIVE

S. Rodríguez[1], S. Balboa[1], G. Olveira[1], J. Montes[2], D. Moreno[3], A. Barrajón[3], J.L. Barja Pérez[1]
[1]CIBUS- Facultad de Biología. Universidade de Santiago de Compostela, Departamento de Microbiología y Parasitología, Santiago de Compostela, Spain
[2]Centro de Investigaciones Marinas, Departamento de Patología, Vilagarcia de Arousa, Spain
[3]Agencia de Medio Ambiente y Agua, Medio Ambiente, Sevilla, Spain

Backgrounds

Large bivalves as *Pinna nobilis* are of remarkable importance in seagrasses fields (mainly *Posidonia oceanica*) along the Mediterranean Sea. Wild populations of this threatened species, considered as "vulnerable" by the spanish law, have been greatly reduced due to abusive fishing and alteration of their habitat. Lately, populations of *Pinna* from Almeria (south-east Spain), are being affected by high mortalities up to 90%, with still unknown origin.

Objectives

To determine the possible cause of the mortalities by means of a multidisciplinary approach including complete microbiota analysis seeking for viral, bacterial and parasitic bivalve pathogens, as well as phytotoxins.

Methods

Adult specimens were aseptically biopsied and samples of mantle, gills, gonad and hepatopancreas were taken. Pieces were fixed for histopathologic analysis. Virus detection was done by PCR and culture methods. For bacterial identification, tissues were spread in TCBS and MA, and representative colonies were isolated. Phenotypic profiles and sequencing of the 16S rRNA gene from a number of isolates were done.

Conclusions

- No parasites of bivalves, as *Perkinsus*, *Marteilia* and *Bonamia* were found.
- Ostreid herpesvirus (OsHV-1), was not detected by PCR, however, IPNV-like was isolated by cell culture, although it is unlikely to be the causative agent of the mortality, since that virus seems not replicates in mollusc tissues.
- Surprisingly, the mollusc pathogen *Vibrio tapetis* was isolated within the high diversity of vibrios characterized.
- However the pathogen that caused the *Pinna nobilis* mortality should be very specific because it does not affect, apparently, to *Pinna rudis* sharing the same niche.

FEMS7-1192
Pathogens / Pathogenicity

DOES ORAL SEX CAUSE FEMALE INFERTILITY?

P. Bavoil[1], P. Marques[1], R. Brotman[2], J. Ravel[3]
[1]University of Maryland - Baltimore, Department of Microbial Pathogenesis, Baltimore, USA
[2]Institute for Genome Sciences, Department of Epidemiology and Public Health, Baltimore, USA
[3]Institute for Genome Sciences, Department of Microbiology and Immunology, Baltimore, USA

Backgrounds

The origin of persistent/resurgent chlamydial infection post-antibiotic treatment is unexplained.

Objectives

To address this question(s), we hypothesize that:

1. The *Chlamydiaceae* have evolved as commensals of the gastrointestinal (GI) tract with fecal-oral transmission (FOT) as the principal route of dissemination to new hosts. Where FOT is reduced (e.g., via sanitation), the occurrence of chlamydiae in the GI tract is reduced.

2. *Chlamydia trachomatis* is a commensal microorganism of the human GI tract, and an opportunistic pathogen in the conjunctiva, genital and respiratory tracts. Where FOT is reduced, direct contact is the primary mode of transmission.

3. *C. trachomatis* is efficiently transmitted to the GI tract of new hosts via oral sex. The practice of oral sex has 'reintroduced' *C. trachomatis* to the human GI tract in communities where FOT was previously reduced.**Methods**

Circumstantial, historical and recent evidence that support the hypothesis is reviewed. Imaging of mCherry-expressing *Chlamydia muridarum* in the murine GI tract was obtained.

Conclusions

Tenets 1 & 2 imply a revision of the status of *C. trachomatis* from principal pathogen to commensal organism that may cause opportunistic infection at non-GI mucosal epithelia. Observed on/off expression of polymorphic membrane proteins, unique properties of peptidoglycan and lipo-oligosaccharide, and extruded inclusions may facilitate chlamydial survival and colonization of the GI tract.

Tenet 3 suggests that orally inoculated chlamydiae may colonize the GI tract, reach the rectum and chronically/episodically, infect the female genital tract causing/contributing to tubal pathology and infertility. The global hypothesis therefore raises the provocative question: does oral sex cause or contribute to female infertility?

FEMS7-2964
Pathogens / Pathogenicity

CLONAL POPULATION STRUCTURE OF MYCOPLASMA HOMINIS TUNISIAN ISOLATES ASSOCIATED WITH GENITAL MANIFESTATIONS

B. Ben Abdelmoumen Mardassi[1]
[1]*Institut Pasteur de Tunis, Mycoplasmas, Belvedere Tunis, Tunisia*

Backgrounds

Mycoplasma hominis is a bacterium that is capable of invasive diseases such as genital infections, adverse pregnancy outcomes and infertility. Little is known on the genetic diversity and pathogenicity of *M. hominis* isolated in Tunisia.

Objectives

The aim of this study was to develop a combined Multi-Locus and Multi- Virulence-Locus Sequence Typing (MLST and MVLST, respectively) scheme for the characterization of *M. hominis* Tunisian isolates.

Methods

The combined MLST-MVLST assay that we developed is based on five housekeeping genes and five virulence genes. These loci were sequenced, aligned, and sequence types (STs) were deduced. This approach was applied to *M. hominis* PG21 reference strain and 55 clinical isolates recovered from the genital tract of Tunisian patients with genital infections and infertility disorders. START2 software was used to determine the Standardized Index of Association (I_A^S). The phylogenetic analysis were inferred using MEGA v6 and SplitsTree v4.0 softwares.

Conclusions

The MLST-MVLST analysis of 55 isolates of *M. hominis* identified 29 STs, with a $I_A^S$of 0,3289 ($p<0,001$), indicating a strong clonal population structure. Phylogenetic analyses further identified two major lineages: Lineage A (17 STs) and Lineage B (12 STs). Interestingly, we noticed that STs from Lineage A were associated with infertility while STs from lineage B were linked to genital infections.

This study uncovered a clonal population structure of *Mycoplasma hominis* Tunisian isolates with a clear distinction between clinical strains causing genital infections and those associated with infertility. ure. Phylogenetic analyses further identified two major lineages: Lineage A (17 STs) and Lineage B (12 STs). Interestingly, we noticed that STs from Lineage A were associated with infertility while STs from lineage B were linked to genital infections.

FEMS7-2076
Pathogens / Pathogenicity

BIOFILM FORMATION POTENTIAL AND TOXIN GENES CONTENT IN STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM AN ALGERIAN HOSPITAL'S ENVIRONMENT

F. Bendali[1]
[1]*Bejaia University, Microbiology, Bejaia, Algeria*

Backgrounds

Staphylococcus (*S.*) *aureus* is a major cause of infections associated with medical devices (urinary catheters, orthopedic implants…). Its pathogenicity is related to an exhaustive arsenal of virulence factors and toxins. Biofilm formation is one of the major pathogenic factors which facilitates adherence to medical devices and depends on different types of adhesins especially the polysaccharide intercellular adhesin (PIA) coded within the *icaADBC* operon.

Objectives

In this study, twenty two (22) *S. aureus* isolates from hospital environment were analyzed to investigate the presence or absence of the *icaA, icaD* genes and their biofilm formation potential.

Methods

Biofilm potential of the strains was investigated by Tissue Culture Plate (TCP) and Congo Red Agar (CRA) methods. The toxinic genes content was determined by multiplex-PCR using specific primers.

Conclusions

All the strains were demonstrated strong biofilm producers in Trypticase Soy Broth (TSB; $0.270 \leq A_{630nm} \leq 0.656$) and only three of them were moderate in Brain Heart Infusion Broth (BHI; $0.170 \leq A_{630nm} \leq 0.237$). Among them, 18 strains were *slime* producers. Their biofilm formation potential was independent of the presence of *icaAD* genes. Investigation of their toxin genes content and antibiotic resistance revealed that almost all the strains harbored at least one of the toxin genes investigated with a high level of resistance to multitude antibiotics. Furthermore, 54 % of the strains were identified as methicillin- resistant *S. aureus* (MRSA). This study showed the high virulence degrees of the *S. aureus* strains which colonize different inert surfaces, susceptible to be present in a health care unit, and could be transferred to hospitalized patients.

FEMS7-2646
Pathogens / Pathogenicity

COMPARISON OF TWO AUTOMATIC PATHOGEN DNA ISOLATION METHODS

K. Benulič[1], T. Cerar Kisek[1], M. Kodre[1], V. Cvitkovic Spik[1], E. Ruzic-Sabljic[1]
[1]Institute of Microbiology and Immunology- Faculty of Medicine- University of Ljubljana, Laboratory for Molecular Bacteriology and Micology, Ljubljana, Slovenia

Backgrounds

Broad-range 16S rRNA PCR/sequencing is a widely used molecular diagnostic method for detecting bacterial pathogens in clinical samples. Clinical samples usually contain smaller amounts of bacterial DNA compared to human DNA, which can also have an inhibitory effect. While commercially available SelectNA plus® (Molzym, Bremen, Germany) facilitates isolation of microbial DNA by degradation of human DNA, MagNA Pure Compact Nucleic Acid Isolation Kit® (Roche, Germany), routinely used in our laboratory, isolates the total DNA present in a specimen.

Objectives

The aim of the study was to evaluate and compare SelectNA plus® and MagNA Pure Compact® nucleic acid isolation methods.

Methods

Nucleic acid was extracted from 85 clinical specimens (19 synovial fluid, 23 pleural effusion, 13 cerebrospinal fluid, 10 abscess content, 6 drainage content, 8 tissue samples, 2 whole blood, 1 pericardial fluid, 2 peritoneal fluid, 1 haematoma) using SelectNA plus® and MagNA Pure Compact® nucleic acid isolation kits. The success of isolation by the two kits was evaluated by performing an in-house 16S rRNA/sequencing method.

Conclusions

Broad-range PCR resulted in 55/85 (64.7%) negative samples with both DNA isolation methods. Both DNA isolation methods yielded positive results in 16/85 (18.8%) samples, in 11/16 samples the identification of the pathogen was identical. The results were concordant in 66 (77.6%) samples. Regarding the number of detected and identified bacteria in our samples, the Magna Pure Compact® method for DNA isolation was better. A considerable advantage of Magna Pure Compact® system is also its convenience and shorter hands-on time.

FEMS7-3246
Pathogens / Pathogenicity

PHENOTYPIC HETEROGENEITY INSIDE THE LEAF: PSEUDOMONAS SYRINGAE WITHIN ITS HOST

N. López-Pagán[1], J.S. Rufián[1], D. López-Márquez[1], J. Ruiz-Albert[1], C.R. Beuzón[1]
[1]Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora IHSM-UMA-CS, Plant Protection, Málaga, Spain

Backgrounds

Advances in genomics and single-cell analysis have demonstrated the extraordinary complexity that microbial populations reach within their hosts. Communities range from complex multispecies groups, to homogeneous populations differentiating through genetic or non-genetic mechanisms. Diversity within populations is recognised as a key driver of evolution in animal pathogens. However, much less is known in plants pathogens about how populations differentiate, the extend of these processes, or how interactions between pathogenic and non-pathogenic variants impacts defence responses or the fate of each variant.

Objectives

To investigate how the model plant pathogen *Pseudomonas syringae* differentiates into subpopulations through non-genetic means, the extent of these processes, or how variation impacts on bacterial adaptation to the plant apoplast, are some of the aims of our work.

Methods

Confocal and time-lapse fluorescent microscopy and flow cytometry have been applied to the analysis of *P. syringae pv. phaseolicola* strains carrying chromosome-located transcriptional fusions to fluorescent reporter genes to genes important for bacterial adaptation to the plant host, as well as to that of bacterial derivatives carrying mutations and/or plasmids affecting regulatory genes.

Conclusions

Non-pathogenic variants of *P. syringae* can proliferate and even spread when in close proximity to pathogenic bacteria. High bacterial concentrations can be reached at natural entry points and promote such interactions during the infection process. But we also found diversity affecting virulence traits originating through non-genetic mechanisms within clonal populations. Our results illustrate the dynamics and complexity of the interactions found within *P. syringa*e infections, and their potential impact on bacterial adaptation

FEMS7-3023
Pathogens / Pathogenicity

A GENOME-WIDE EPIDEMIOLOGICAL STUDY OF PSEUDOMONAS AERUGINOSA IN NORTHEAST ITALY

I. Bianconi[1], *O. Jousson*[1]
[1]*University of Trento, Centre for Integrative Biology CIBIO, Trento, Italy*

Backgrounds

Pseudomonas aeruginosa is a ubiquitous bacterium which can be found in soil and aquatic environments and is able to colonize plants, animals and humans. *It* has a characteristic non-clonal population structure consisting of a limited number of widespread major clones that are highly versatile in their habitat and of minor clones with preference for a peculiar niche. While patient-to-patient transmission is well documented, the role of the environment as a reservoir of initial infectious clones is still unclear and may lead to inadequate preventive measures. Moreover, a better understanding of *P. aeruginosa* transmissibility between different environments and the human host is important to develop more targeted therapies against the pathogen.

Objectives

To date, no systematic epidemiological studies of *P. aeruginosa* have been conducted in Italy. We are therefore performing a cross-sectional genomic epidemiological study to provide an overview of *P. aeruginosa* population structure in Northeast Italy and to investigate the circulation of strains between human and animal hosts and the environment.

Methods

*S*trains have been collected from water, soil, animal and human hosts and their genome will be sequenced and analysed.

Conclusions

Whole genome sequencing and population studies will allow (i) to elucidate the source of new strains causing infections in humans; (ii) to determine if major clones are present within the population and to analyse their distribution among the different hosts and environments; (iii) to verify the presence in Northeast Italy of putative epidemic lineages and track their mode of transmission.

FEMS7-1010
Pathogens / Pathogenicity

PREDICTING ANTIBIOTIC RESISTANCE BY EXPERIMENTAL EVOLUTION IN STENOTROPHOMONAS MALTOPHILIA

P. Blanco Torres[1], *J.L. Martínez Menéndez*[1]
[1]*Centro Nacional de Biotecnología- CSIC, Microbial Biotechnology, Madrid, Spain*

Backgrounds

Stenotrophomonas maltophilia is an opportunistic pathogen with increasing prevalence characterized by a reduced susceptibility to currently used antibiotics. Such resistance is mainly due to the presence of antibiotic-inactivating enzymes and several multidrug efflux pump (MDR) systems encoded in its genome. However, less is known about the mutations involved in the acquisition of resistance of this bacterium that are selected in the presence of antibiotics.

Objectives

The aim of our study was to predict the emergence of resistance in *S. maltophilia* during 28 days of experimental evolution in the presence of tigecycline or ceftazidime and to determine the mutations involved. The order of appearance of each of the mutations is of relevance for predicting the contribution of each independent mutation to antibiotic resistance.

Methods

A stepwise experiment was performed in the presence of increasing inhibitory concentrations of each antibiotic, starting at minimum inhibitory concentration (MIC) and stopping at 32MIC. Evolution was also performed at subinhibitory concentrations, using 1/10 and 1/50 MIC continuously. Controls without any compound were also grown in parallel. At the end of the experimental evolution, four independent populations of each treatment were taken for whole-genome sequencing (WGS). At several time points, samples were taken to establish the order of appearance of each mutation by PCR amplification and Sanger sequencing.

Conclusions

High levels of resistance have been obtained in all cases at inhibitory concentrations of both antibiotics; however, at subinhibitory concentrations no changes in the MICs were recorded. Different mutations that will be discussed in the presentation, account for the observed phenotype.

FEMS7-0605
Pathogens / Pathogenicity

THE ANTIFUNGAL PHENOTYPE OF MYELOID CELLS DIFFERENTIATED FROM HEMATOPOIETIC PROGENITORS WITH M-CSF, BUT NOT WITH GM-CSF, IS ENHANCED BY CANDIDA ALBICANS IN A TLR2-DEPENDENT MANNER

C. Bono[1], A. Martínez[1], J. Megías[2], D. Gozalbo[1], M.L. Gil[1]
[1]Universitat de Valencia,
Microbiología y Ecología and Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina E RI BIOTECMED, Valencia- Burjassot, Spain
[2]Universitat de Valencia, Patología, Valencia, Spain

Backgrounds

In vitro recognition of *C. albicans* by hematopoietic stem and progenitor mouse cells (purified as lineage negative or Lin^- cells) promotes differentiation toward macrophages. Additionally, the antifungal phenotype of macrophages obtained in the presence of M-CSF is enhanced by *C. albicans* [1].

Objectives

Our objectives were to determine the involvement of TLR2 in this phenotype and to elucidate whether fungal recognition also modulates the antifungal properties of GM-CSF-differentiated myeloid cells.

Methods

GM-CSF-differentiated mature myeloid cells obtained from Lin^- cells showed an increased ability to kill *C. albicans*, as compared with cells differentiated with M-CSF. Co-incubation of Lin^- cells with GM-CSF and *C. albicans* yeasts did not modify: (i) the ability of the differentiated cells to kill yeasts and (ii) the cytokine production (TNF-alpha and IL-6) in response to pure TLR ligands. Interestingly, myeloid cells obtained from Lin^- progenitors of TLR2 KO mice, showed identical killing ability regardless the presence or absence of *C. albicans* during their differentiation with M-CSF.

Conclusions

Recognition of *C. albicans* by Lin^- cells affects differentially the antifungal phenotype of derived mature myeloid cells in a cytokine-dependent manner. Fungal killing of M-CSF-differentiated cells is enhanced by *C. albicans* in a TLR2-dependent manner, whereas this effect is not observed when Lin^- cells are differentiated with GM-CSF.

[1] Megías *et al*. (2016). Microbes and Infection 18(5):354-63.

This work was supported by grant SAF2014-53823-P (Ministerio de Economía y Competitividad, Spain).

FEMS7-3150
Pathogens / Pathogenicity

MYCOPLASMA BOVIS ISMBOV1 AND ITS DERIVATIVES ARE CONSERVED GENETIC ELEMENTS OF RNA POLYMERASE ALTERNATIVE SIGMA FACTOR GENOMIC LOCUS

I. Lysnyansky[1], *I. Borovok*[2]
[1]*Kimron Veterinary Institute, Avian and Aquatic Diseases, Beit Dagan, Israel*
[2]*Tel Aviv University, Molecular Microbiology and Biotechnology, Tel Aviv, Israel*

Backgrounds

Analysis of *Mycoplasma bovis* genomic data determined genes encoding two RNA polymerase sigma factors. One is a putative primary RpoD-like subunit, and another one resembles alternative sigma factors of the ECF (Extracytoplasmic Function) family.

Objectives

Revealing genomic organization of the gene cluster encoding alternative sigma factor.

Methods

Standard bioinformatics tools including blast algorithms, sequence alignments, and phylogeny were used to mine and analyze available *M. bovis* genomes.

Conclusions

While the *rpoD* locus has the same organization in analyzed *M. bovis* genomes, an alternative sigma factor operon reveals the differences of its regulatory region. One or two copies of IS*Mbov1* are located immediately upstream of the *cysS-spoU-sigECF-rpmG-secE-nusG* operon. When two IS*Mbov1* copies coexist, they may perform different arrangements (e.g. two copies have the same or an opposite orientation). *M. bovis* strains of different geographic origins have always one defective IS*Mbov1* upstream of *cysS*. Internal deletions within an IS*Mbov1* transposase gene suggest that this IS element is dysfunctional. The reference *M. bovis* strain PG45 has two IS*Mbov1* copies located upstream of *cysS* - a distal (relatively to *cysS*) copy carries a transposase pseudogene, while a proximal IS copy encodes an intact transposase. Genomes of several Chinese *M. bovis* isolates have only a defective copy of IS*Mbov1* upstream of *cysS*. Comparative analysis of geographically distinct strains revealed that a defective IS*Mbov1* copy upstream of *cysS* shares a high DNA sequence conservation (an average identity >95%) suggesting a putative role of this IS*Mbov1* remnant in regulation of the downstream located operon encoding an alternative sigma factor.

FEMS7-0553
Pathogens / Pathogenicity

GENETIC AND BIOCHEMICAL DISSECTION OF SYSTEM DEDICATED TO THE ACQUISITION AND THE UTILIZATION OF N-ACETYLGLUCOSAMINE IN THE PHYTOPATHOGENIC BACTERIUM XANTHOMONAS

A. Boulanger[1,2], S. Dupoiron[3], L. Ligat[4], M. Arlat[1,2], L. Noël[1], E. Jamet[5], C. Albenne[6], E. Lauber[1]
[1]INRA-CNRS UMR441/2594, LIPM- Plant-Microorganism interaction laboratory, CASTANET TOLOSAN, France
[2]Toulouse University Paul Sabatier -UPS, Microbiology, Toulouse, France
[3]INRA/URCA FARE, Fractionnement des agroressources et environnement, Reims, France
[4]INSERM, CRCT-Toulouse Center for cancer research, Toulouse, France
[5]CNRS-UPS UMR 5546, LRSV- Plant Science research laboratory, Toulouse, France
[6]CNRS-UPS UMR5100, CBI-LMGM Molecular genetic and microbiology laboratory, Toulouse, France

Backgrounds

N-acetylglucosamine (GlcNAc) is an amino sugar important for a broad range of organisms ranging from bacteria to plants and animals. Because GlcNAc is only present in trace amounts in plants, it has not previously been considered as a possible substrate exploited by phytopathogenic bacteria during infection. *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of black rot, infects a wide range of Brassicaceae crop plants and expresses a carbohydrate utilization system devoted to GlcNAc exploitation to be characterized.

Objectives

Characterize organization, regulation and roles of the *Xcc* GlcNAc system.

Methods

We used a combination of methods including molecular genetics, pathoassays, mass spectrometry, and biochemistry to decrypt this GlcNAc utilization pathway.

Conclusions

In addition to genes involved in GlcNAc catabolism, this system codes for four TonB-dependent outer membrane transporters (TBDT), nine glycoside hydrolases (GH) and two transcriptional repressors. Expression of all these genes is induced in presence of GlcNAc. This regulon confer to *Xcc* the ability to exploit chitooligosaccharides that could originate from fungi or insects, to recycle *Xcc*-derived peptidoglycan/muropeptides and to metabolize plant GlcNAc-containing molecules during infection. *In vitro* analyses also demonstrate that five of the nine GHs are involved in the sequential degradation of a plant *N*-glycopeptide. Altogether these results extend the range of sources of GlcNAc metabolized by *Xcc* during its life cycle. This is the first evidence of GlcNAc consumption and *N*-glycan degradation during infection by a phytopathogenic bacterium, a feature shared with human pathogenic bacteria.

FEMS7-1256
Pathogens / Pathogenicity

EFFECT OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) VACCINATION ON TUBERCULOSIS IN WILD BOAR

M. Bravo[1], D. Risco[2], R. Martínez[1], T. Almudena[1], J.M. Cuesta[3], P. Gonçalves[2], W.L. García Jiménez[4], R. Cerrato[2], R. Iglesias[5], A. Quesada[5], L. Gómez[3], P. Fernández-Llario[2], J. Hermoso de Mendoza[1]
[1]Universidad de Extremadura, Departamento de Sanidad Animal, Cáceres, Spain
[2]Innovación en Gestión y Conservación de Ungulados SL,
Innovación en Gestión y Conservación de Ungulados SL, Cáceres, Spain
[3]Universidad de Extremadura, Departamento de Medicina Animal, Cáceres, Spain
[4]University of Surrey, Department of Pathology and Infectious Diseases, Guilford- Surrey, United Kingdom
[5]Universidad de Extremadura, Departamento de Bioquímica y Biología Molecular y Genética, Cáceres, Spain

Backgrounds

Recently, there has been evidence showing that coinfections with porcine circovirus type 2 (PCV2) may favour higher prevalences and the development of more severe tuberculosis pattern in wild boar populations. Therefore, measures focused on controlling PCV2 could be useful for reducing the impact of tuberculosis in this species.

Objectives

The objective of this study is to evaluate the effect of PCV2 vaccines on the prevalence and lesional severity of tuberculosis in wild boar populations.

Methods

The study was developed in a fenced estate in which two groups of wild boars were generated: a group of animals that were captured, identified by transponder and vaccinated against PCV2 (n = 36); and a control group (n = 48), that was not vaccinated. The animals of both groups lived in the same habitat until a percentage of them were captured in hunting events celebrated in December of 2013, 2014 and 2015. Clinical and microbiological diagnoses was performed to determine which of these animals suffered from tuberculosis and, if appropriate, their lesional pattern (localized or generalized). These parameters were compared between the two groups using statistical tests.

Conclusions

Our preliminary results suggest that vaccination against PCV2 may be a valuable measure to reduce the severity of tuberculosis lesions in wild boar, since a lower percentage of animals with generalized lesions was found among the vaccinated group.

This vaccination did not significantly reduce the percentage of infected animals. Potentially longer period of time would be necessary to observe any effect on the prevalence of tuberculosis.

FEMS7-0354
Pathogens / Pathogenicity

ATTENUATED MYCOBACTERIUM TUBERCULOSIS VACCINE SECRETING DTP ANTIGENS: BASIS FOR A MULTIVALENT PROTECTION

E. Broset[1], A.I. Kanno[2], L.C.C. Leite[2], C. Martin[1], J. Gonzalo-Asensio[1]
[1]Universidad de Zaragoza, Microbiología- Medicina Preventiva- y Salud Pública, Zaragoza, Spain
[2]Instituto Butantan, Centro de Biotecnologia, São Paulo, Brazil

Backgrounds

BCG is al live attenuated vaccine effective in reducing severe forms of tuberculosis (TB) in children but confers variable protection against pulmonary TB in adults. To overcome this handicap, we have developed the live vaccine MTBVAC currently in phase Ib clinical trials in South Africa (NTC02729571).MTBVAC is based on the attenuation of *Mycobacterium tuberculosis* by inactivation of the *phoP* and *fadD26* virulence genes.

Objectives

Because its adjuvant capacity, BCG has been traditionally proposed as vector to deliver several antigens. In this context, we propose the study of MTBVAC as a multivalent recombinant vaccine against different infectious diseases including diphtheria, tetanus and whooping cough, which actually forming DTP vaccine.

Methods

In order to construct a multivalent MTBVAC, genetically inactivated and codon optimized genes for diphtheria (CRM197), pertussis toxoids (S1), or Fragment C of tetanus toxin (FC) were synthesized. Each gene was cloned under the control of a new mycobacteriophage L5 derived promoter. To lead the secretion of these antigens, the signal sequence of Ag85A (a known mycobacterial secreted protein) was placed in frame before each antigen. Protein expression and secretion of CRM197, S1 and FC was confirmed by Western blot and mass spectrometry.

Conclusions

Preliminary experiments in mice showed that vaccination with rMTBVAC expressing S1 toxoid protects against *Bordetella pertussis* colonization. On-going experiments aimed to detect anti-DTP antibodies in mice vaccinated with these rMTBVAC. Taking together, these results provide *proof-of concept* that MTBVAC is an efficient vector to deliver heterologous antigens and put forward rMTBVAC to protect against different infectious diseases.

FEMS7-2865
Pathogens / Pathogenicity

THE HUMAN URINARY MICROBIOME IN BLADDER CANCER

V. Bučević Popović[1], *M. Šitum*[2], *V. Punda Polić*[3], *J. Terzić*[4]
[1]*Faculty of Science- University of Split, Department of Chemistry, Split, Croatia*
[2]*Clinical Hospital Split, Department of Urology, Split, Croatia*
[3]*School of Medicine- University of Split, Department of Microbiology, Split, Croatia*
[4]*School of Medicine- University of Split, Department of Immunology, Split, Croatia*

Backgrounds

Although traditionally considered sterile, recent studies have shown that bladder, similar to other body sites, harbours a diverse microbial population which could be a contributing factor to urinary bladder pathogenesis. Most of the studies published so far were analysing females, proposing an association of female urinary microbiota with disorders such as urgency urinary incontinence and urinary tract infection. Knowledge of male urinary microbiota is very limited, and the link between urinary microbiota and bladder cancer, known to be three times more common in men then in women, has not been explored yet.

Objectives

The main goal of our study was to examine male urinary microbiome in bladder cancer patients and compare it with urinary microbiome of healthy controls.

Methods

Urine samples were obtained from male bladder cancer patients and healthy controls. DNA was isolated from urines and enriched for bacterial 16S V4 rDNA region by PCR amplification. The amplicons were sequenced on Illumina MiSeq platform and data analysis was performed in collaboration with Second Genome's Microbial Profiling Service.

Conclusions

The urine microbiome from bladder cancer patients and healthy controls did not exhibit significant alternations at a microbial community level. Operational taxonomic units that differed significantly between the two groups included the genera *Kocuria*, *Actinobaculum*, *Fusobacterium*, *Micrococcus*, *Pyramidobacter* and *Corynebacterium* which were more abundant in bladder cancer, and the genera *Streptococcus* and *Veillonella*, enriched in healthy samples. Further studies with larger cohort are needed to establish the role of urinary microbiota in bladder cancer development and treatment response.

FEMS7-3191
Pathogens / Pathogenicity

CROSS-STRESS BEHAVIOR OF GENERAL PORINS IN YERSINIA PSEUDOTUBERCULOSIS UNDER PROLONGED ANTIBIOTIC EXPOSURE

E. Bystritskaya[1,2], *A. Stenkova*[1,2], *A. Rakin*[3], *M. Isaeva*[1,2]
[1]*G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Laboratory of Marine Biochemistry, Vladivostok, Russia*
[2]*Far Eastern Federal University, Biomedical School, Vladivostok, Russia*
[3]*Max von Pettenkofer Institute for Hygiene and Clinical Microbiology, Yersinia Phylogenomics Laboratory, Munich, Germany*

Backgrounds

One of survival strategy for bacteria confronted with unfavorable conditions such as antibiotics is altering membrane permeability via expression of general porins. During prolonged exposure to antibiotics, the bacteria encounter many other stresses caused by stationary phase (starvation, pH change, high metabolite concentration). These factors can provide cross-protection against antibiotic stress. Little is known about how reciprocally regulated porins affect the adaptive response of *Y. pseudotuberculosis* to antibiotics under multiple stresses.

Objectives

Our aim was to determine if general porins are essential for adaptive behavior of *Y. pseudotuberculosis* to a wide spectrum of stress conditions.

Methods

We investigated porin gene expression in *Y. pseudotuberculosis* 488 exposed to sublethal concentrations of different classes of antibiotics using real-time RT-PCR and GFP-based flow cytometry analyses.

Conclusions

We found no significant changes in *ompC* and *ompF* transcription, indicating that the stationary-phase stress strongly regulated the porin expression balance and protected from sublethal antibiotic treatment. Results with GFP-reporter strains were more complex. *ompF* was predominant in all investigated unfavorable conditions, probably playing more important role in protective bacterial strategy. This gene was strongly downregulated under 37°C, however remaining at higher level compared to *ompC*. Tetracycline and nalidixic acid slightly decreased expression of *ompC*. Carbenicillin treatment led to the appearance of heterogeneously fluorescent bacterial subpopulations. We believe that the survival of *Y. pseudotuberculosis* under prolonged antibiotic exposure is not due to porin deficiency, but is associated with other resistance mechanisms provided by environmental cross-stresses.

FEMS7-1237
Pathogens / Pathogenicity

ADDING FUNCTION TO THE GENOMES OF GLOBAL AND AFRICAN SEQUENCE TYPES OF SALMONELLA TYPHIMURIUM

R. Canals[1], D. Hammarlöf[2], C. Kröger[3], S.V. Owen[1], W.P.M. Rowe[1], R.R. Chaudhuri[4], K. Hokamp[5], M.A. Gordon[6], J.C.D. Hinton[1]
[1]Institute of Integrative Biology- University of Liverpool, Department of Functional and Comparative Genomics, Liverpool, United Kingdom
[2]Uppsala University, Department of Cell and Molecular Biology /Microbiology, Uppsala, Sweden
[3]Moyne Institute of Preventive Medicine- Trinity College Dublin, Department of Microbiology, Dublin, Ireland
[4]University of Sheffield, Department of Molecular Biology and Biotechnology, Sheffield, United Kingdom
[5]Smurfit Institute of Genetics- Trinity College Dublin, Department of Genetics, Dublin, Ireland
[6]University of Liverpool, Institute of Infection & Global Health, Liverpool, United Kingdom

Backgrounds

Salmonella Typhimurium infects a wide range of animal hosts, and generally causes a self-limiting gastroenteritis in humans. New variants of this serovar, sequence-type ST313, are associated with an emerging invasive nontyphoidal *Salmonella* (iNTS) disease in sub-Saharan Africa that targets immunocompromised HIV^+ or malarial individuals. We studied an ST313 isolate, D23580, and the well-characterized isolate 4/74 (sequence-type ST19) that causes gastroenteritis across the globe. A genomic comparison showed that the two strains share 96% of coding genes, and genetic differences included 1000 SNPs, two D23580-specific prophages, a different plasmid repertoire, and the presence of pseudogenes.

Objectives

We hypothesized that the two strains had different pathogenic mechanisms, and searched for altered gene expression patterns and gene fitness in infection-relevant environmental conditions.

Methods

RNA-seq-based transcriptomic data were obtained for strains 4/74 and D23580, grown under sixteen infection-relevant *in vitro* conditions and during infection of murine macrophages. Key transcriptomic data were validated with a proteomic approach. In addition, transposon insertion sequencing was used to identify genes in the D23580 strain required for growth in infection-relevant conditions, and intracellular survival in macrophages.

Conclusions

Differences observed in gene expression of virulence-associated genes under specific environmental conditions reflect the distinct pathogenic mechanisms of these two *S*. Typhimurium strains. We will present our latest results involving the analyses of transcriptomic, proteomic and transposon insertion sequencing data.

FEMS7-2560
Pathogens / Pathogenicity

UNCOMMON VIRULENCE POTENTIAL IN ST-14 KPC-3 KLEBSIELLA PNEUMONIAE: A HIGH RISK CLONE IN PORTUGAL

C. Caneiras[1], L. Lito[2], J. Melo-Cristino[2,3], A. Duarte[1]
[1]Research Institute for Medicines iMed.ULisboa - Faculty of Pharmacy - University of Lisbon, Department of Microbiology and Immunology, Lisboa, Portugal
[2]Centro Hospitalar Lisboa Norte, Laboratory of Microbiology, Lisboa, Portugal
[3]Institute of Molecular Medicine - Faculty of Medicine - University of Lisbon, Institute of Microbiology, Lisboa, Portugal

Backgrounds

The emergence of KPC-type carbapenemases in *Klebsiella pneumoniae* poses a serious threat to public health worldwide and have recently undergone a relevant increasing prevalence in Portugal. Studies regarding the *K. pneumoniae* identification of virulence markers and high-risk clones are critical for the development of novel diagnostic methods and new therapeutic strategies.

Objectives

The aim of this study was the identification of virulence markers and characterization of the clonal relationship among multidrug resistant *Klebsiella pneumoniae* isolates producing KPC-3 carbapenemases since 2009, in Portugal.

Methods

This study included 27 representative clinical isolates of *K.pneumoniae* KPC-3 producers collected in a tertiary hospital centre in Lisboa, Portugal, between 2009 and 2013. Antimicrobial susceptibility testing was performed by disk diffusion and the results were interpreted according to CLSI and EUCAST guidelines. Genes encoding other β-lactamases including OXA, NDM, CTX-M, TEM, SHV, DHA, FOX, and CMY were screened by PCR and confirmed by sequencing. The isolates were also screened for gene markers of virulence factors: *K2A, fimH, mrkD$_{V1}$, mrkD$_{V2-4}$, khe, rmpA, magA,* and *iucC* by PCR amplification.The clonal relationship was evaluated by M13 fingerprinting and multilocus sequence typing (MLST).

Conclusions

We firstly report an uncommon and concerning overlapping of multidrug-resistance and accumulation of virulence genes in the prevalent (>80%) ST-14 clone identified. The combination of the KPC-3 gene with virulence genes as K2 capsular serotype, fimbrial adhesins, haemolysin, and aerobactin - a bacterial iron chelating agent, can constitute a serious threat, especially for vulnerable populations and may further exacerbate infections caused by this pathogen.

FEMS7-0875
Pathogens / Pathogenicity

POLYAMINE MOLECULES TARGETING EPIGENETIC EFFECTORS IN THE EXPRESSION PROFILES OF NONRIBOSOMAL PEPTIDE SYNTHETASES GENE FAMILY IN THE PHYTOPATHOGEN FUNGUS BOTRYTIS CINEREA

F.M. Ana[1], G.R. Victoria E.[1], G. Carlos[1], C. Maria[1], B. Alejandro[1], J.M. Cantoral Fernandez[1]
[1]Universidad De Cadiz, Biomedicina- Biotecnologia Y Salud Publica. Lab. Microbiologia, Puerto Real, Spain

Backgrounds

Botrytis cinerea is a phytopathogen fungus that shows an interesting secondary metabolism. It involves the biosynthesis of a broad range of cell wall--degrading enzymes, toxins, polyketides and fatty acid-derived compounds, terpenes and nonribosomal peptides and amino acid-derived compounds.

Nonribosomal peptide synthetases (NRPS) are a group of enzymes that play important roles in fungal metabolisms. They are related with siderophores biosynthesis and obtaining ion metal from the environment. Genome of *B. cinerea* contains nine NRPS encoding genes but the role of them is unknown to this day.

Polyamines are a family of chemical compounds in relation, between other, with the biosynthesis of siderophores in microorganisms. Several studies have proved the importance of polyamines in the host-pathogen interaction during the development of disease causing by *B. cinerea*.

Objectives

This work shows how the presence of polyamines affects to the expression profiles of NRPS gene family in *B. cinerea*.

Methods

Culture mediums are supplemented with no-lethal concentration of four polyamines: 1,3-diaminopropane, spermidine, spermine and suberoylanilide-hydroxamic acid. *B. cinerea* B05.10 grows during 22 days with six sample-times. After, expression profiles are studied by RT-qPCR.

Conclusions

Expression of these genes is clearly modified in the presence of polyamines. Depending of their chemical structure, genes are upregulated or downregulated in comparison with control condition. This study provides interesting data about how this chemical compound can produce the expression of gene that were silenced in normal growth of *B. cinerea*, opening a door to future metabolomic studies in the secondary metabolisms of this important fungus.
Project founded by MINECO: AGL2015-65684-C2-2-R and FEDER.

FEMS7-3158
Pathogens / Pathogenicity

CHARACTERIZATION OF THE RHIZOFERRIN BIOSYNTHETIC GENE IN THE FUNGAL PATHOGEN, RHIZOPUS DELEMAR

C. Carroll[1], C. Grieve[1], I. Murugathasan[1], C. Czekster[2], H. Lui[2], J. Naismith[2], M. Moore[1]
[1]Simon Fraser University, Biological Sciences, Burnaby, Canada
[2]University of St. Andrews, Biomedical Sciences Research Complex, St. Andrews, United Kingdom

Backgrounds

Iron is essential for growth and in low iron environments, some microbes secrete ferric iron (Fe^{3+})-chelating molecules called siderophores. All fungi produce hydroxamate siderophores with the exception of Mucorales fungi; these secrete the polycarboxylate siderophore, rhizoferrin. At present, the importance of rhizoferrin biosynthesis to Mucorales pathogenesis is unknown.

Objectives

To identify and characterize the enzyme responsible for biosynthesis of rhizoferrin by the pathogenic fungus, *Rhizopus delemar*.

Methods

A putative rhizoferrin synthetase (*rfs*) gene belonging to the NRPS-independent siderophore (NIS) family was identified in the genome of *R. delemar* 99-880. qPCR confirmed that expression of the putative *rfs* gene was repressed by iron. *rfs* was cloned and expressed in *E. coli* and the Rfs protein was purified using affinity chromatography. Siderophore biosynthesis by recombinant Rfs from citrate and diaminobutane, the two building blocks of rhizoferrin, was confirmed using high resolution LC-MS/MS. Rfs also used the substrate derivatives ornithine, oxaloacetic acid, hydroxylamine, diaminopentane and diaminopropane, and produced mono-citryl intermediates for all derivatives except tricarballylic acid. Di-citryl compounds were detected with all derivatives except oxaloacetic acid. Site-directed mutagenesis studies showed that histidine 444 is required for Rfs activity, while leucine 544 may play a role in governing amino-substrate specificity.

Conclusions

Rfs catalyzes rhizoferrin biosynthesis in a two-step manner; first, activating citrate through adenylation and second, condensing the citryl intermediate with a nucleophile. We propose that Rfs is a new fungal member of the superfamily of adenylating enzymes. Studies are underway to assess the effect of *rfs* in virulence of Mucorales fungi.

FEMS7-0485
Pathogens / Pathogenicity

EFFECT OF VACCINATION AGAINST ORAL HPV INFECTION IN WOMEN IN HIGH SCHOOLS FROM CALI, COLOMBIA

A. Castillo[1], A. Arbelaez[2], J.C. Osorio[2], L.E. Bravo[3]
[1]Universidad del Valle, Department of Biology- Faculty of Exact and Natural Sciences, Cali, Colombia
[2]Universidad del Valle, School of Biomedical Sciences, Cali, Colombia
[3]Universidad del Valle, Cali Cancer Registry- Department of Pathology, Cali, Colombia

Backgrounds

Human papillomavirus (HPV) is responsible for an increasing proportion of oropharyngeal squamous cell cancers. However, it is not clear whether the vaccine for HPV, to prevent cervical cancer, could provide protection against oral HPV infections.

Objectives

A risk/prevalence study that examines the relationship between HPV vaccination and HPV oral detection in 300 women vaccinated for HPV.

Methods

Oral cell samples were collected in 300 women aged between 14 and 17 years old who had been vaccinated for HPV, and in 76 women who had not been vaccinated. The procedure to obtain the samples consisted in 15-second rinse and 15-second gargle with 15 ml mouthwash. DNA extraction was made using DNeasy Blood & Tissue Kit (QIAGEN) and the HPV DNA detection by GP5+/GP6+ primers set-mediated PCR. Statistics produced include the mid-p exact tests, and risk/prevalence ratio (relative risk) that were performed using the OpenEpi statistics program (http://www.openepi.com/TwobyTwo/TwobyTwo.htm).

Conclusions

HPV detection in women vaccinated was 2 out 300 (0.7%), and in women without a vaccine was 5 out 76 (6.6%). The mid-P exact results were less than 0.01, which suggest that there is an association. The Risk Ratio (RR) was 0.1013, a modest effect indeed (5.9% decrease in risk), with 95% confidence limits barely excluding 0 or 1.
The study shows a modest association between vaccination for HPV and a decreasing of HPV oral infection. Our results suggest the need to develop community-level strategies of information and education to encourage HPV vaccination in girls in Cali, Colombia

FEMS7-2848
Pathogens / Pathogenicity

ISOLATION AND CHARACTERISATION OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI FROM NORWEGIAN BLUE MUSSELS (MYTILUS EDULIS)

C. Cedillo Martin[1], C.S. Svanevik[2], G. Johannessen[3], C. Sekse[4], B.T. Lunestad[2]
[1]University of Bergen, Department of Biology, Bergen, Norway
[2]National Institute of Nutrition and Seafood Research, Seafood safety and health, Bergen, Norway
[3]Norwegian Veterinary Institute, Bacteriology - Food and GMO, Oslo, Norway
[4]Norwegian Veterinary Institute, Bacteriology - Aquatic and Terrestrial Animals, Oslo, Norway

Backgrounds

There have been no outbreaks by Shiga toxin-producing *E.coli* from blue mussels, and only few studies concerning STEC detection in coastal environments and bivalves have been reported.

Objectives

To improve our understanding of STEC, presence of STEC was investigated in 270 blue mussels collected from harvesting areas along the Norwegian coast in 2016.

Methods

Microbial enrichment of the blue mussel samples followed by DNA extraction for screening of STEC-associated genes was performed as described in ISO/TS -13136. Real-time PCR assays were conducted for genes encoding Shiga toxin (*stx*), intimin (*eae*) and the five major European serogroups (O157, O26, O111, O145 and O103). Isolation of STEC was attempted from samples with positive PCR result for *stx* and *eae* by plating the enriched samples and picking of 50 colonies. Colonies were screened for presence of *stx* and positive isolates were further characterized to determine their serotype and virulence profile. For two samples immunomagnetic separation (IMS) was performed to facilitate isolation of STEC. Up to 30 colonies per serogroup were selected for identification and the isolates were further characterised.

Conclusions

The screening results revealed the presence of the virulence genes (*eae* and *stx*) in the 19 blue mussel samples. *stx* positive isolates were isolated from four of these samples as described above. Presumptive colonies from different serogroups were identified from the IMS and confirmed by real-time PCR for each serogroup. This preliminary data indicates a low prevalence and therefore low risk of human infection by STEC if bivalves from these harvesting areas were to be consumed.

FEMS7-3112
Pathogens / Pathogenicity

NEW SEQUENCE TYPES OF N. GONORRHOEAE IN KENYA REVEAL HIGH PREVALENCE OF MOBILE GENETIC ELEMENTS

A. Cehovin[1], O. Harrison[2], S. Lewis[1], P. Ward[1], S. Lea[1], E. Sanders[3], M. Maiden[2], C. Tang[1]
[1]University of Oxford, Sir William Dunn School of Pathology, Oxford, United Kingdom
[2]University of Oxford, Department of Zoology, Oxford, United Kingdom
[3]Kemri-Wellcome Trust Research Program, Kemri, Kilifi, Kenya

Backgrounds

The sexually transmitted infection gonorrhoea caused by *Neisseria gonorrhoeae* is a major public health concern, associated with significant morbidity worldwide. In Africa, the incidence rates are the highest in the world but little is known about the strains causing gonococcal disease in adults.

Objectives

Characterisation of a collection of gonococcal strains from high-risk individuals in Kenya by:

- determining their multi-locus sequence types (MLSTs) and antimicrobial resistance (AMR) profiles,

- studying genealogical associations between MLST and conjugative plasmid types,

- determining whether repeat infections are due to relapse or re-infection with a different strain.

Methods

The gonococcal strains were sequenced and annotated in PubMLST.org/Neisseria, which annotates defined loci. To determine MLSTs and AMR profiles, we defined the core genome and AMR loci of strains, respectively. We then used gene-by-gene comparison to resolve population relationships and elucidate the sites of genetic variation.

Conclusions

Gonococcal strains in coastal Kenya belong to new MLSTs that are distinct from those found in other parts of the world. Compared to strains circulating in the UK and US, this gonococcal population harbours high prevalence of conjugative plasmids conferring tetracycline resistance. Genealogical plasmid associations indicate that each lineage is associated with a distinct plasmid backbone. In particular, genetic variation between lineages occur in *tetM* and a toxin-antitoxin system. In addition, our gene-by-gene comparison reveals that repeat gonococcal infections are due to re-infections with different strains. This analysis provides insights into the gonococcal population in Kenya and plasmid evolution among gonococcal strains.

FEMS7-1137
Pathogens / Pathogenicity

COMPARATIVE GENOMICS OF CLINICAL AND ENVIRONMENTAL SAMPLES OF CAMPYLOBACTER JEJUNI

D. Cejkova[1], M. Medvecky[1], Z. Sekelova[1], O. Polansky[1], T. Kubasova[1], K. Simunovic[2], S. Smolte Mozina[2], I. Rychlik[1]
[1]Veterinary Research Institute, Department of Immunology, Brno, Czech Republic
[2]Biotechnical Faculty- University of Ljubljana, Department of Food Science and Technology, Ljubljana, Slovenia

Backgrounds

Campylobacteriosis is major bacterial food-borne disease in industrialized countries in recent years with a large economic burden. The major causative agent of human campylobacteriosis is *Campylobacter jejuni* which accounts for 90% of human cases. *C. jejuni* is considered as a member of commensal microbiota in birds, thus the raw or undercooked poultry products are the major sources of human cases. However, *C. jejuni* strains can also survive in water and waterborne outbreaks of campylobacteriosis are also widely and frequently reported.

Objectives

In order to identify genotypes associated with clinical and environmental sources, we determined and compared the complete genome sequences of 15 *C. jejuni* isolates originating from water sources (5 isolates) and human beings (10 isolates).

Methods

Extracted DNA from each isolate was subjected to DNA sequencing on the Illumina MiSeq and NextSeq platforms, followed by *de novo* read assembly via IDBA-UD software and *ab initio* gene prediction using the Prokka pipeline.

Conclusions

The total pan-genome of sequenced isolates comprises 2345 proteins, whereas core-genome consists of 1371 proteins. Comparing genomes of human and water isolates, thirty-six proteins were only associated with human isolates. These human-associated proteins were mainly involved in sugar, amino and fatty acid metabolism, transport, iron uptake and cell wall biosynthesis. On contrary, water-associated proteins included eight hypothetical proteins. In addition, human isolates showed intra-strain variation in homopolymeric tract length in genes coding for flagellar and other motility proteins. These data indicate that human isolates of *C. jejuni* are more virulent and showed increased adaptation to a novel host.

FEMS7-2035
Pathogens / Pathogenicity

METHYLENE BLUE AS AN EFFECTIVE PHOTOACTIVE DYE IN ERADICATION OF RESISTANT BIOFILMS FORMED BY PATHOGENIC YEASTS OF CANDIDA PARAPSILOSIS AND CANDIDA ALBICANS

L. Černáková[1], S. Dižová[1], H. Bujdáková[1]
[1]Comenius University in Bratislava- Faculty of Natural Sciences,
Department of Microbiology and Virology, Bratislava, Slovak Republic

Backgrounds

Treatment of biofilm formed by *Candida* species on medical devices is limited. Therefore, current research is searching for new options, like photodynamic inactivation (PDI). PDI works through activation of photoactive dye (methylene blue – MB) with the light of appropriate wavelength resulting in reactive oxygen species production.

Objectives

This study was focused on effectiveness of PDI compared to caspofungin (CAS) on biofilms formed by *C. parapsilosis* and *C. albicans.*

Methods

Two light sources were used for irradiation: red LED light (1.67 mW/cm^2) or laser (190 mW/cm^2). Inhibition of sessile biofilm cells was considered to be effective when reduction was minimally 50% ($SMIC_{50}$) compared to the control sample without MB. Two sets of experiments were performed when MB was added to the adherence phase or to the pre-formed 24-h biofilm. Experiments were evaluated after 48 and 72 h, respectively.

Conclusions

Results proved that application of CAS to the adherence phase can prevent biofilm development ($SMIC_{50}$ was 1 µg/ml for *C. parapsilosis* and 0.13 µg/ml for *C. albicans*), while an addition of this compound (16 µg/ml) to the 24-h biofilm was not efficient. Determination of colony forming units and XTT reduction assay confirmed efficacy of MB (1 mmol/l) used in PDI on different biofilm stages. Moreover, microscopy of biofilms formed on mouse tongues sections (*ex vivo* model, 72 h) explicitly confirmed previous observations. Both light sources were effective, but laser enabled a decreasing period of irradiation while maintaining the high effectiveness of PDI. MB used for PDI can combat fully developed resistant biofilms.

FEMS7-0266
Pathogens / Pathogenicity

HOW PROTEASES PRODUCED BY ENTEROCOCCUS FAECALIS PROTECT IT AGAINST THE SHORT A-HELICAL ANTIMICROBIAL PEPTIDES

V. Čeřovský[1], O. Nešuta[1], M. Buděšínský[2], R. Hadravová[3], R. Hexnerová[4]
[1]Institute of Organic Chemistry and Biochemistry - Academy of Sciences of the Czech Republic, Antimicrobial Peptides, Prague 6, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry - Academy of Sciences of the Czech Republic, NMR and Molecular Spectroscopy, Prague 6, Czech Republic
[3]Institute of Organic Chemistry and Biochemistry- Academy of Sciences of the Czech Republic, Biochemistry and Molecular Biology, Prague 6, Czech Republic
[4]Institute of Organic Chemistry and Biochemistry - Academy of Sciences of the Czech Republic, Structural Biology, Prague 6, Czech Republic

Backgrounds

Enterococcus faecalis is a common Gram-positive bacterium colonizing the gastrointestinal tract and oral cavity of animals and humans. As an opportunistic pathogen, it causes life-threatening nosocomial infections because of its frequent multidrug resistance. Its resistance to antimicrobial peptides (AMPs) resides in the secretion of two proteases - gelatinase (GelE) and serine protease (SprE) able to degrade them.

Objectives

We have focused on understanding the role of these two proteases in the degradation of short α-helical AMPs called HYL-20 (GILSSLWKKLKKIIAK-NH_2) and 14-NH_2 (KRLFKELKFSLRKY-NH_2).

Methods

Cleavage sites in HYL-20 and 14-NH_2 after the incubation with *E. faecalis* were identified using RP-HPLC fractionation and MS analysis of fragments. We used 1,10-phenanthroline, a specific GelE inhibitor, to determine contribution of both proteases to the degradation. In addition, an analogous peptide with D-Lys at its C-terminus (HYL-20*k*) was used as a substrate to study a role of C-terminal de-amidation step in the process. We also studied the effect of secondary structure of HYL-20 and HYL-20*k* determined by NMR spectroscopy on their antimicrobial activity.

Conclusions

The C-terminal deamidation of HYL-20 is the first step of peptide degradation by *E. faecalis* making the peptide susceptible to consecutive cleavage by GelE at internal peptide bonds, which finally results in deterioration of its antimicrobial activity. This is attributed to an unexpected and not yet unidentified protease which also cleaves internal peptide bonds of AMPs at hydrophobic residues. In the contrast to published data participation of SprE in the protective mechanism of *E. faecalis* against AMPs was not proved.

FEMS7-1904
Pathogens / Pathogenicity

STUDY OF BARTONELLA PREVALENCE AMONG WILD AND DOMESTIC ANIMALS IN GEORGIA

N. Chakvetadze[1], N. Abazashvili[1], B. Ying[2], L. Malania[1], M. Kosoy[2]
[1]National Center for Disease Control and Public Health, Science, Tbilisi, Georgia
[2]Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Fort Collins, USA

Backgrounds

Bartonella species are intracellular bacteria of erythrocytes and endothelial cells. The bacteria in the genus *Bartonella* are zoonotic pathogens of wild and domestic animals.

Objectives

Our objective was to detect the presence of *Bartonella* species in animals in Georgia.

Methods

During 2015-2016 had been collected 382 animal samples, among which 151 were rodents, 81-cattle, 116 - dogs, 34 - sheep, captured from 7 districts of Georgia. Bacteriological investigation of all samples had been conducted as well as molecular typing of isolated cultures.

We identified 29 rodent-associated *Bartonella* species (*B. elizabethae, B. tribocorum, B. grahamii*) by culturing from rodent samples – 19.20%; from cattle samples had been isolated 23 B. bovis and 3 B. schonbuchensis (32%), 5 isolates of *Bartonella vinsonii* subsp. *berkhoffii* had been isolated from dog samples (4.3%) and no bacterial growth was detected among sheep. *Bartonella* isolates were confirmed by polymerase chain reaction (PCR) as well as cultures were further genetically characterized by sequence analysis of five chromosomal regions 16S rRNA, gltA, ftsZ, rpoB, and the intergenic spacer region (ITS).

Conclusions

On the basis of these data, we propose that findings in Georgia only indicating the highest diversity of *Bartonella* spp. These results have important public health implications, because identified *Bartonella* species have been reported as pathogenic bacteria for human.

These data will allow us to build a comprehensive picture of the risks associated with exposure to *Bartonella* species.

We are grateful to the International Science and Technology Center for the financial support.

FEMS7-0856
Pathogens / Pathogenicity

NATURALLY OCCURRING INLB VARIANTS WITH A DIFFERENT POTENTIAL TO CAUSE PERINATAL LISTERIOSIS IN MICE.

Y. Chalenko[1], S. Ermolaeva[2], K. Sobyanin[3]
[1], Moscow, Russia
[2]Center, Gamalei Center of Microbiology and Epidemiology, Moscow, Russia
[3]Gamalei center of Microbiology and Epidemiology, laboratory of ecology pathogens, Moscow, Russia

Backgrounds

The Gram-positive bacterium Listeria monocytogenes causes listeriosis, a severe disease with multiple manifestations including stillbirths and meningitis of newborns. The invasion factor of the internalin family InlB I involved in crossing the maternal-fetal barrier (Disson et al., 2008). Previously, we described several InlB variants that differed in the ability to support intragastric infection in mice Sobyanin et al., 2017).

Objectives

To compare effects of InlB variants on perinatal infection in mice.

Methods

The InlB variants differing in 10 amino acid substitutions were expressed under the same promoter in the *L. monocytogenes* strain EGDeΔinlB. Work with animals was performed with approval of local bioethical committee. Mice were intragastrically infected on the 14th day of pregnancy, euthanized 1 and 3 dpi, bacterial loads were determined by plating.

Conclusions

One of two InlB variants provided infection of both placentas and fetuses while another did not. Bacteria were revealed in placentas 24 and 72 hpi. Infection was unequal for different fetuses in the same animal. Obtained results suggested that some InlB variants might promote perinatal infection in mice.

Disson et al. Nature. 2008, 455:1114-1118.

Sobyanin et al. FEMS Miscrobiol Lett. 2017. in press

FEMS7-0480
Pathogens / Pathogenicity

IMMUNOGENICITY PROFILING OF PROTEINS FROM CAPSULAR GROUP B NEISSERIA MENINGITIDIS

C.M. Chang[1], A. Awanye[1], L. Marsay[2], C. Dold[2], C. Rollier[2], A. Pollard[2], J. Derrick[1]
[1]Faculty of Biology- Medicine and Health, Division of Infection- Immunity and Respiratory Medicine, Manchester, United Kingdom
[2]Oxford Vaccine Group, Department of Paediatric, Oxford, United Kingdom

Backgrounds

Outer membrane vesicle (OMV) based vaccines have been used to provide strain-specific protection to serogroup B *Neisseria meningitidis* infection. The identification of immunogenic outer membrane proteins (OMPs) may help to develop a vaccine with broader strain coverage.

Objectives

A meningococcal OMPs microarray panel was manufactured and used to determine the highly immunogenic antigens. Differences in IgG responses in human and mouse were compared.

Methods

Ninety-three OMPs were selected based on a proteomic study of OMVs. OMPs were individually expressed, purified, and refolded before printed on the slides. IgG responses were determined using antisera from vaccinated mice, or adults who had received an OMV vaccine in a Phase I Clinical Trial (PMCID: PMC4535279). For human antisera, IgG binding was quantified at pre-immunization, and two post-immunization stages. Highly immunogenic antigens in mouse and human were determined based on the magnitude and significance of responses between pre- and post-immunizations.

Conclusions

All antigens with the highest responses to human IgG were integral OMPs (iOMPs) but, in mice, the proportion of iOMPs and soluble proteins was more evenly distributed. The repertoire of highly responding antigens in human and mouse was similar, but not identical. The dominance of responses to iOMPs in humans emphasizes the importance of these antigens in the immunoresponse to OMV vaccination. This result raises the importance of maintaining protein conformational integrity, particularly for integral membrane proteins. Additionally, our work shows that protein antigen microarray panels can be a valuable asset to study responses to vaccines which contain complex mixtures of antigens, such as OMVs.

FEMS7-0699
Pathogens / Pathogenicity

COMPARISON OF O-POLYSACCHARIDE AND HEMOLYSIN CO-REGULATED PROTEIN AS TARGET ANTIGENS FOR SERODIAGNOSIS OF MELIOIDOSIS

N. Chantratita[1]
[1]*Faculty of Tropical Medicine - Mahidol University, Microbiology and Immunology, Bangkok, Thailand*

Backgrounds

Melioidosis is a severe disease caused by *Burkholderia pseudomallei*. Clinical manifestations are diverse and acute infections require immediate treatment with effective antibiotics. While culture is the current diagnostic standard, it is time-consuming. A lack of good serodiagnostic tools can impede diagnosis and disease surveillance. Recent studies have suggested that O-polysaccharide (OPS) and hemolysin co-regulated protein 1 (Hcp1) are promising target antigens for serodiagnosis of melioidosis.

Objectives

We evaluated rapid ELISAs using crude antigens, purified OPS and Hcp1 to measure antibody levels in three sets of sera: (i) 419 serum samples from melioidosis patients, Thai and U.S. healthy donors, (ii) 120 serum samples from patients with other bacterial infections, and (iii) 423 serum samples from 200 melioidosis patients obtained upon admission and at 12 and 52 weeks post-recovery.

Methods

We observed significantly higher antibody levels using the crude antigen prepared from wild type *B. pseudomallei* compared to that of an OPS-mutant. The areas-under-receiver-operator-characteristics (AUROCCs) for diagnosis were compared for individual Hcp1-ELISA or OPS-ELISA or combined Hcp1/OPS-ELISA. For Thai donors, AUROCCs were highest and comparable between the Hcp1-ELISA and the combined Hcp1/OPS-ELISA (0.95 versus 0.94). For U.S. donors, the AUROCC was highest for the combined Hcp1/OPS-ELISA (0.96). Significantly higher seropositivity was observed in diabetic patients compared to those without diabetes for both the Hcp1-ELISA (87.3% versus 69.7%) and OPS-ELISA (88.1% versus 60.6%). Although antibody levels for Hcp1 were highest upon admission, the titers declined by week 52 post-recovery.

Conclusions

Hcp1 represents a promising target antigen for the development of POC tests for acute melioidosis.

FEMS7-0925
Pathogens / Pathogenicity

SURVIVAL AND BIOFILM FORMATION OF ACINETOBACTER PITTII UNDER UNFAVORABLE CONDITIONS RESEMBLING HOSPITAL SETTINGS

I. Chapartegui Gonzalez[1], *M. Lazaro Diez*[1], *Z. Bravo Del Hoyo*[1], *J. Ramos Vivas*[1]
[1]*IDIVAL, Microbiology, Santander, Spain*

Backgrounds

The importance of the gram-negative rod *Acinetobacter* as the cause of nosocomial infections has increased during the last years. *A. pittii* is an opportunistic pathogen frequently isolated from *Acinetobacter* infections. It was recognized that at least some *A. baumannii* strains are able to survive for long periods under dry conditions, increasing its probability of causing infections in the hospital settings. However, the survival of *A. pittii* on inanimate surfaces under unfavorable environmental conditions is unknown.

Objectives

The aim of the present work was to study both the survival capacity and the biofilm formation ability of six *Acinetobacter pittii* clinical isolates under desiccation conditions, on surfaces typical of those found in the hospital environment (plastic, glass and lab coat) up to 6 weeks.

Methods

For this work we selected 6 *Acinetobacter pittii* clinical isolates. Viable counts present on inert surfaces under unfavorable conditions were determined up to 43 days, by colony counting. Also, cell viability was determined with a Live/Dead staining and CLS Microscopy. Biofilm formation ability along time was assessed in 24-well plates by crystal violet staining.

Conclusions

Survival and biofilm formation capacities of *A. pittii* were strain-dependent.
In all isolates a significant decline of culturable count took place after desiccation, although for some strains, a viable cell subpopulation is maintained beyond 6 weeks. No significant differences between surfaces were observed. Live/Dead staining corroborated these results. Importantly, our results showed an overall tendency to increase the biofilm formation over time, suggesting that unfavorable environmental conditions may trigger virulent phenotypes.

FEMS7-3125
Pathogens / Pathogenicity

IDENTIFICATION OF A COMMON TRANSCRIPTION FACTOR REGULATING VIRULENCE AND ANTIBIOTIC RESISTANCE: A KEY STRATEGY TO EVADE MDR AND UPEC PATHOGENESIS

A.K. Chaurasia[1], N. Batool[1], R. Subramaniyam[1], K.K. Kim[1]
[1]School of Medicine- Sungkyunkwan University, Department of Molecular Cell Biology, Suwon, Republic of Korea

Backgrounds

Rapid emergence of multiple drugs resistant (MDR) pathogenic bacteria through clonal selection is one the major setback of indiscriminate uses of antibiotics. Recent reports on resistance against last-resort antibiotics, colistin in uropathogenic *Escherichia coli* (*UPEC*), raised an urgent need to devise a novel strategy to inhibit virulence and drug resistance.

Objectives

Since the establishment of successful infection is a favorable tilt of offense/defense of host and pathogen towards pathogen. Therefore, supporting the host innate immune response by targeting conceptual virulence and antibiotics responsive transcription factor (VarTF) of pathogens may prove as a cornerstone strategy to evade MDR and pathogenesis of *UPEC*.

Methods

By employing multinomics approach, we identified a hypothetical transcription factor *c0879* (*varTF*) and its downstream regulatory network, derepressed during infection. The expression of VarTF is controlled by non-canonical G-quadruplex structure in its divergent promoter (P_{c0879}) which was experimentally verified G4 specific signature peak and melting temperature. The deletion mutant of *varTF* does not show any physiological disadvantage in rich media while the overexpression of VarTF showed antibiotic susceptibility and extremely reduced invasion/survival potential in macrophage compared to wild type. RNA sequencing data showed VarTF regulates a cascade of virulence and antibiotics resistance genes involved in hemolysin activity, cell-division proteins and nuclear occlusion factor, hydroperoxidase I, iron II uptake and gtutaredoxin etc. Moreover, VarTF acts as a repressor for ArcA, SlyA and Fur transcriptional regulators responsible for micro-aerobic environment adaptation, bacterial survival in macrophage and ferric uptake repressor, respectively.

Conclusions

The repression of hydroperoxidase I and consequent accumulation of hydrogen peroxide; and excessive uptake of iron II are expected to favor Fenton reaction to produced highly reactive oxygen and nitrogen species, subjecting *UPEC* cells susceptible to both host innate immune system and chloramphenicol including other antibiotics.

FEMS7-0526
Pathogens / Pathogenicity

DELETION OF SDHA INFLUENCES TYPE THREE SECRETION SYSTEM OF ESCHERICHIA COLI O157:H7

W.C. Cheng[1], H.Y. Tasi[1], C.S. Chen[2], J.W. Chen[3], S.T. Hu[1], W.J. Syu[1]
[1]National Yang-Ming University, Microbiology and Immunology, taipei, Taiwan R.O.C.
[2]National Cheng Kung University, Biochemical and Molecular Biology, Tainan, Taiwan R.O.C.
[3]National Cheng Kung University, Microbiology and Immunology, Tainan, Taiwan R.O.C.

Backgrounds

Enterohaemorhagic *Eschericia coli* (EHEC), one virotype of the pathogenic *E. coli,* causes hemorrhagic colitis and sometimes renders hemolytic uremic syndrome. EHEC can inject effector proteins into host cells via type-3 secretion system (T3SS) and consequently induce pathological alterations on the infected host cells. Metabolic gene *sdhA* that codes for succinate dehydrogenase subunit A has been shown to affect the EHEC virulence in the *Caenorhabditis elegans* model. SdhA is one of the enzymes in TCA cycle and catalyzes the reaction of succinate into fumarate.

Objectives

We aimed at knowing the possible effect of *sdhA* on the expression and secretion of T3S proteins.

Methods

To study the influence of *sdhA* on T3S, we cultured an EHEC wild-type strain (EDL933) and its *sdhA*-deleted mutant in M9 aerobically, with the presence of 5% CO_2, or anaerobically without O_2 and then separated the culture into supernatant and bacterial pellet. Representative T3S proteins in these two fractions were detected by Western blotting, and, subsequently, the differences between the *sdhA* knockout and the parental strain were compared. Besides the *sdhA*-deleted strain, we also examined these proteins expressed and secreted from mutants with other genes disrupted in the TCA cycle.

Conclusions

We observed that deletion of *sdhA* enhanced T3S proteins secretion and expression aerobically while this deletion induced selective T3S proteins secreted anaerobically. The phenomena were also seen with other TCA gene-disrupted mutants, but not with all.

FEMS7-0330
Pathogens / Pathogenicity

REACTIVE OXYGEN SPECIES DRIVE THE EVOLUTION OF PRO-BIOFILM VARIANTS IN PATHOGENS BY MODULATING CYCLIC-DI-GMP LEVELS

S.L. Chua[1], *L. Yang*[2]
[1]*Nanyang Technological University, Lee Kong Chian School of Medicine, Singapore, Singapore*
[2]*Nanyang Technological University, SCELSE, Singapore, Singapore*

Backgrounds

The host immune system offers a hostile environment with antimicrobials and reactive oxygen species (ROS) that are detrimental to bacterial pathogens, forcing them to evolve for survival. For instance, *Pseudomonas aeruginosa* can survive and form host-adapted variants in cystic fibrosis lungs, despite the presence of host immune system and antibiotics treatment. While the evolution of bacteria to antibiotics was well-studied, the contribution of oxidative stress by immune system to pathogen evolution remains elusive.

Objectives

[1] Study the evolution of pathogens to resistance of oxidative stress and identify the mutations important in the resistance of oxidative stress by DNA sequencing

[2] Elucidate the mutations-associated mechanisms, leading to the resistance of oxidative stress

[3] Study the relevance of evolved variants in macrophage infection

Methods

Using an experimental evolution strategy, we show that exposure of *P. aeruginosa* to sub-lethal hydrogen peroxide (H2O2) levels over 120 generations led to the emergence of pro-biofilm rough small colony variants (RSCVs), which could be abrogated by L-glutathione antioxidant. Comparative genomic analysis of the RSCVs revealed that mutations in the *wspF* gene, which encodes for a repressor of WspR diguanylate cyclase (DGC), were responsible for increased intracellular cyclic-di-GMP content and production of Psl exopolysaccharide. Psl provides the first line of defence against ROS and macrophages, ensuring the survival fitness of RSCVs over wild-type *P. aeruginosa*.

Conclusions

Our study demonstrated that ROS is an essential driving force for the selection of pro-biofilm forming pathogenic variants. Understanding the fundamental mechanism of these genotypic and phenotypic adaptations will improve treatment strategies for combating chronic infections.

FEMS7-0785
Pathogens / Pathogenicity

HARNESSING THE INNATE IMMUNITY IN THE TREATMENT OF STAPHYLOCOCCUS EPIDERMIDIS BIOFILM FORMATION

P.Y. Chung[1], R. Khanum[2], B.Y. Chin[3]
[1]International Medical University, Department of Pathology- School of Medicine, Kuala Lumpur, Malaysia
[2]International Medical University, School of Postgraduate Studies, Kuala Lumpur, Malaysia
[3]International Medical University, School of Health Sciences, Kuala Lumpur, Malaysia

Backgrounds

The alarming rate of bacterial resistance to antibiotics is one of the major challenges to global public health, as antibiotics resistance has been found in all classes of antibiotics used in clinical practice. Of particular concern are the opportunistic and multidrug-resistant pathogens known as ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) that commonly cause nosocomial infections in indwelling medical devices, wound sites and bacteremia. A major virulence factor in these ESKAPE pathogens is biofilm formation, whereby the cells attach irreversibly on various biotic and abiotic surfaces, in an encased hydrated matrix of exopolymeric substances. In addition to the ESKAPE pathogens, multidrug-resistant and biofilm-forming *Staphylococcus epidermidis* has emerged in the recent years as one of the most important global nosocomial and opportunistic pathogen. Thus, it is of paramount importance that more effective treatments for biofilm-associated infections are discovered. One of the strategies to prevent and eradicate biofilm formation which has shown potential is the activation of antimicrobial peptide (AMP), which is an important component of the innate immune system of the host.

Objectives

To determine the activities of lactoferrin, an endogenous AMP with broad antimicrobial spectrum, against reference multidrug-resistant and biofilm-forming *S. epidermidis*

Methods

The biomass of the biofilm and metabolic activity of planktonic and biofilm cells of *S. epidermidis* upon exposure to lactoferrin were determined using standard microtiter plate assays.

Conclusions

Lactoferrin has been shown to eradicate biofilms of *S. epidermidis*, while the metabolism of the bacteria in the biofilms was reduced.

FEMS7-2033
Pathogens / Pathogenicity

CANDIDA ALBICANS VS. CANDIDA DUBLINIENSIS: THE COMPARISON OF ADHERENCE PROPERTIES AND BIOFILM DEVELOPMENT IN VITRO, IN VIVO, AND EX VIVO

J. Chupáčová[1], *H. Bujdáková*[1]
[1]*Comenius University in Bratislava- Faculty of Natural Sciences,*
Department of Microbiology and Virology, Bratislava, Slovak Republic

Backgrounds

Candida albicans and *Candida dubliniensis* are closely related organisms in respect to morphology and phylogeny, but with different virulence and pathogenicity.

Objectives

This research compared adhesion properties and biofilm development of *C. albicans* and *C. dubliniensis*, and studied role of biofilm-associated genes (*ALS1*, *ALS9*) in these processes. For this study, *in vitro*, *ex vivo* (mouse tongues), and *in vivo* (*Galleria mellonella* host model) experiments were performed.

Methods

The kinetic of adhesion was studied by metabolic activity (XTT reduction assay) of cells adhered to surface of microtiter plate in selected time points (0, 30, 60, 90, 120 min). Metabolic activities of the matured 48-h biofilms were determined as well. In *ex vivo* experiments, cryo-cuts from mouse tongues were evaluated after PAS staining. *In vivo* model *G.mellonella* was selected for comparison of pathogenicity of biofilms formed by both *Candida* species. Additionally, the RT-PCR was performed in order to determine regulation of the *ALS1*and *ALS9* genes in adhesion and matured biofilm.

Conclusions

Results revealed that in spite of strong ability of *C. dubliniensis* to adhere during adhesion phase (XTT assay), the matured biofilm was thinner, with decreased penetration to the tissue, and composed mainly of the yeast cells (light microscopy and Confocal Laser Scanning Microscopy). Survival of larvae was higher (up to 30%) when they were infected with *C. dubliniensis* compared to the *C. albicans* infection. The *ALS1* and *ALS9* genes seem to be regulated during biofilm development.

Despite of higher fitness of *C. albicans*, *C. dubliniensis* confirmed enough “power” in biofilm development in all models.

FEMS7-0616
Pathogens / Pathogenicity

EVALUATION OF UTP-GLUCOSE-1-PHOSPHATE URIDYLYLTRANSFERASE (UDPG:PP) AS A POTENTIAL NOVEL DRUG TARGET IN STREPTOCOCCUS PNEUMONIAE

F. Cools[1], B. Vanhoutte[1], E. Torfs[1], M. Bidart de Macedo[1], L. Bonofilgio[2], D. Cappoen[1], M. Mollerach[2], P. Cos[1]
[1]University of Antwerp, Laboratory of Microbiology- Parasitology and Hygiene LMPH, Antwerp, Belgium
[2]Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica- CONICET, Microbiología, Buenos Aires, Argentina

Backgrounds

The most important virulence factor of the human pathogen *Streptococcus pneumoniae* is its polysaccharide capsule, which prevents opsonization by complement factors, adhesion and macrophage phagocytosis. Uridine diphosphate glucose (UDP-Glc) is a key component in the biosynthetic pathway of capsular polysaccharides and is also present in other bacteria where it plays a role in lipopolysaccharide and capsule production. It is formed out of glucose 1-phosphate (Glc-1P) by the enzyme UTP-glucose-1-phosphate uridylyltransferase (UDPG:PP), which is encoded by the *galU* gene. UDPG:PP is widely distributed amongst animals, plants and other microorganisms, but eukaryotic UDPG:PPs are evolutionary unrelated to their prokaryotic counterparts. Therefore, it is postulated that UDPG:PP might be a valuable novel target in fighting bacterial diseases.

Objectives

To assess the potential value of UDPG:PP in antimicrobial therapy, several *in vitro* characteristics and the *in vivo* infectivity of different pneumococcal *galU* knockout strains were compared with those of their non-mutated parent strains.

Methods

In vitro data on biofilm formation, antimicrobial susceptibility and co-cultures with macrophages and epithelial cells were generated using standard methods. Transmission electron microscopy was used to visualize the capsule and *in vivo* infectivity was determined using a *Galleria mellonella* model.

Conclusions

Although there is no definitive correlation found for all strains and their knockouts, our results suggest that *galU* mutations influence *in vitro* biofilm formation. Furthermore, results of cellular co-cultures combined with the primary results of infectivity imply UDPG:PP might indeed be a potential new target.

FEMS7-0451
Pathogens / Pathogenicity

MODELLING PATHOGEN INTERACTIONS WITHIN THE BOVINE RESPIRATORY TRACT USING A THREE-DIMENSIONAL DIFFERENTIATED AIRWAY EPITHELIAL CELL MODEL

D. Cozens[1], E. Sutherland[1], G. Taylor[2], C. Berry[3], R. Davies[1]
[1]University of Glasgow, Infection- Immunity and Inflammation, Glasgow, United Kingdom
[2]Pirbright Institute, Virology, Pirbright, United Kingdom
[3]University of Glasgow, Institute of Molecular Cell and Systems Biology, Glasgow, United Kingdom

Backgrounds

Bovine respiratory disease (BRD) complex is a multifactorial infection of cattle that is responsible for substantial economic losses to the livestock industries. Various viral and bacterial agents are associated with BRD although *Mannheimia haemolytica* is one of the major bacterial pathogens involved. The complex interactions involving bacteria, viruses and the host are poorly understood; this is partly due to the lack of physiologically-relevant and reproducible *in vitro* models and the reliance on experiments involving cattle.

Objectives

The aim of the present study was to optimize a three-dimensional differentiated airway epithelial cell (AEC) model of the bovine respiratory tract for studying host-pathogen interactions involved in BRD.

Methods

Primary bovine bronchial epithelial cells, isolated from fresh abattoir material, were cultivated at an air-liquid interface to stimulate differentiation into typical airway epithelial cells. Differentiation was compared under different growth conditions and at different time points. The AEC model was subsequently used to compare infection by different strains of *M. haemolytica* isolated from diseased and healthy cattle.

Conclusions

The AEC model displayed tight junction formation and mucocilary activity, hallmarks of the respiratory tract epithelium. Optimum growth conditions and time-points were determined for infection studies. Strains of *M. haemolytica* isolated from diseased cattle successfully colonised the epithelial layer and caused significant tissue damage by 48 h; conversely, commensal strains isolated from healthy animals were incapable of colonisation. Thus, the AEC model represents a very useful tool for studying the interactions of *M. haemolytica* with the bovine respiratory tract and will have wider applications.

FEMS7-3214
Pathogens / Pathogenicity

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF MULTIDRUG RESISTANT ACINETOBACTER BAUMANNII FROM NEONATAL FEEDING TUBES IN NEONATAL INTENSIVE CARE UNITS

K. Dahmani[1], M. Saad[1], P. Ogrodzki[1], T. Osaili[2], J. Winter[1], S. Forsythe.[1]
[1]Nottingham Trent University, School of Science and Technology, Nottingham, United Kingdom
[2]Jordan University of Science and Technology, Nutrition and Food Technologyhool, Irbid, Jordan

Backgrounds

Acinetobacter baumannii is one of the most opportunistic pathogens responsible for serious infections in intensive care units (ICUs).

Objectives

The aim of this study was to profile a diverse clinical collection of *A. baumannii* by applying a range of phenotyping and genotyping methods to determine the potential infection risk to neonates.

Methods

Forty-three clinical strains had been isolated from neonatal feeding tubes from two neonatal ICUs. They were genotyped using pulsed-field gel electrophoresis using *ApaI* and *AscI* restriction enzymes. The isolates were identified by sequence analysis of the *rpoB* allele (350 bp). Representative strains were subjected to MLST analysis. Isolates were examined for potential, virulence factors, antibiotic resistance, biofilm production, human serum resistance, acid resistance and ability to attach to and invade Caco-2 cells.

All strains were identified as *A. baumannii* and clustered into five pulsotypes. MLST analysis revealed 2 different STs (ST193 and ST113). The majority of strains were resistant to all β-lactam antibiotics tested, but susceptible to ciprofloxacin. blaOXA-51 and blaOXA-64 genes were present in all of the isolates whereas blaOXA-23 was only found in strains belonging to ST113. The majority of the strains showed the ability to form significant biofilms, tolerance of acidic condition and resistance to human serum. All strains testes attached strongly to intestinal cells (Caco-2), except for an ST193 strain which showed low attachment.

Conclusions

The *A. baumannii* strains revealed remarkable virulence factors. These features could allow them to survive in the host and persist in hospitals where neonates are at high risk.

FEMS7-0505
Pathogens / Pathogenicity

LOSS OF THE FUR REGULATOR RESULTS IN DISTINCT PHENOTYPES FOR SALMONELLA ENTERICA SEROVARS TYPHIMURIUM AND TYPHI

J.M. Leclerc[1], C.M. Dozois[2], F. Daigle[1]
[1]Université de Montréal, Microbiology, Montreal, Canada
[2]Institut Armand-Frappier.INRS, Microbiology, Laval, Canada

Backgrounds

Salmonella enterica serovars Typhimurium and Typhi are two closely related bacteria that cause different types of infection in humans. The global regulator Fur, involved in iron homeostasis, stress response and virulence, is present and conserved in both serovars.

Objectives

To investigated the role of Fur in *S.* Typhimurium and *S.* Typhi during growth, motility, infection of macrophages and to determine the total amount of siderophores produced.

Methods

Growth curves of the *fur* mutants under iron-rich and iron-limiting conditions revealed that only the *S.* Typhi *fur* mutant was defective. Also, formation of filamentous cells was only observed in this mutant. Fur was required for optimal swimming motility in both serovars, but motility was more markedly reduced for the *S.* Typhi *fur* mutant. Fur was also important for *S*. Typhi during interaction with human cultured macrophages. The *fur* mutant showed severe defects in uptake and survival within these cells. By contrast, loss of Fur had no effect on *S*. Typhimurium infection of macrophages. Finally, *S*. Typhi produced more siderophores than *S*. Typhimurium when grown to stationary phase in iron-free medium and both serovars produced more enterobactin than salmochelins.

Conclusions

These results demonstrate that Fur differentially affects the physiology and the virulence phenotypes of the two serovars and is more critical for *S*. Typhi growth, morphology, motility and interaction with host cells than for *S*. Typhimurium.

FEMS7-3241
Pathogens / Pathogenicity

THE GUT PATHOBIONT HELICOBACTER HEPATICUS PRODUCES A LARGE POLYSACCHARIDE INDUCING A MSK/CREB-DEPENDENT ANTI-INFLAMMATORY GENE SIGNATURE IN MACROPHAGES

C. Danne[1]
[1]*Kennedy Institute, NDORMS, Oxford, United Kingdom*

Backgrounds

Shaped by co-evolution, host-microbiota mutualism depends on intricate molecular interactions. However, the identities of symbiotic molecules promoting tolerance, and the immune mechanisms that discriminate between the microbiota and infection-causing pathogens remain elusive. Especially as persistent colonizers can sometimes behave as pathobionts.

H. hepaticus is a common member of the mouse gut microbiota, but it induces colitis when the IL-10 signaling is blocked. The model of IBD using *H. hepaticus* and an anti-IL10R antibody shares many cardinal features of human IBD. IL10-producing Treg cells induced by *H. hepaticus* were shown to prevent inflammation in this model. However, little is known about *H. hepaticus* interactions with the innate immune compartment.

Objectives

We then investigated wether *H. hepaticus* could promote intestinal homeostasis.

Methods

We observed that *H. hepaticus* colonization induces IL-10 production in gut-resident macrophages and bone-marrow derived macrophages (BMDMs). Interestingly, we identified a large soluble polysaccharide produced by *H. hepaticus* that induces IL-10 *in vitro* and *in vivo*, along with a low pro-inflammatory response. We then showed that *H. hepaticus* polysaccharide signals through TLR2/MyD88. A microarray analysis revealed the specific anti-inflammatory/repair gene signature induced by *H. hepaticus* polysaccharide in BMDMs. By contrast, the canonical TLR2/1 ligand Pam3CSK4 induces a strong pro-inflammatory/activation response. The immunomodulatory properties of *H. hepaticus* polysaccharide are dependent on MSK1/2 and the transcription factor CREB, with a low induction of NF-kB.

Conclusions

A better understanding of the molecular crosstalk between cells and microbes is of major importance to decipher mutualism, with the ultimate goal to propose new preventive and therapeutic strategies.

FEMS7-0610
Pathogens / Pathogenicity

A SMALL MOLECULE CANDIDATE FOR ANTIBIOTIC CO-THERAPY IN THE FIGHT AGAINST BACTERIAL PERSISTENCE

V. Defraine[1], V. Liebens[1], R. Corbau[2], A. Marchand[2], P. Chaltin[2,3], F. Van Bambeke[4], A. Anantharajah[4], M. Fauvart[1,5], M. Jan[1]
[1]KU Leuven, Centre for Microbial and Plant Genetics, Leuven, Belgium
[2]KU Leuven, Centre for Drug Design and Discovery, Heverlee, Belgium
[3]CISTIM, Leuven vzw, Leuven, Belgium
[4]Université Catholique de Louvain,
Pharmacologie cellulaire et moléculaire- Louvain Drug research Institute, Brussels, Belgium
[5]imec, Department of Life Science Technologies, Leuven, Belgium

Backgrounds

Bacterial infections pose a major public health threat and are predicted to cause 10 million deaths annually by 2050. This is mainly driven by rapidly increasing multidrug resistance and the presence of antibiotic-tolerant persister cells that greatly impede disease treatment.

Objectives

In the search for novel antibacterial strategies we discovered a novel small molecule that significantly decreases the surviving fraction of *Pseudomonas aeruginosa* in combination with ofloxacin. A multi-pronged approach was used to further characterize SPI009 and gain more insight into the mode of action.

Methods

Extensive antibacterial assays, using both SPI009 alone and in combination with either β-lactam, aminoglycoside, polymyxin or fluoroquinolone antibiotics successfully eradicated cultures of different Gram-negative and Gram-positive bacteria, including the ESKAPE pathogens and several (multidrug resistant) clinical isolates of *P. aeruginosa.* Testing of SPI009 in different model systems revealed potent biofilm inhibition and eradication while combination treatment with ciprofloxacin resulted in the efficient eradication of an intracellular *P. aeruginosa* infection in human monocytes. Different approaches were used to study the mode of action of SPI009, including macromolecular synthesis assays, membrane permeability studies and comparative genomic analysis of mutants with differing susceptibility. All pointed towards extensive membrane damaging activity of SPI009, leading to either direct cell death or facilitation of antibiotic-induced cell death.

Conclusions

Crucially, due to the observed broad-spectrum effect, antibiotic-independent activity and possibility to tackle persister cells, SPI009 provides a promising starting point for the development of novel co-therapies in the fight against multidrug-resistant and persistent infections.

FEMS7-0243
Pathogens / Pathogenicity

BURKHOLDERIA BACTERIA AS A SOURCE OF NOVEL ANTIMICROBIALS

E. Depoorter[1], E. De Canck[1], T. Coenye[2], P. Vandamme[1]
[1]Ghent University, Department of Biochemistry and Microbiology, Gent, Belgium
[2]Ghent University, Department of Pharmaceutical Analysis, Gent, Belgium

Backgrounds

Because of the urgent need for new antibiotics and the low efficiency of high-throughput screens, it is time to reconsider drug discovery from natural sources. *Burkholderia* bacteria have large genomes with extensive potential for secondary metabolism and they are well-known for their metabolic diversity and biocontrol properties. The recent discovery that strains of *B. ambifaria*, *B. gladioli* and *B. vietnamiensis* produce antibiotics with activity against multidrug resistant pathogens, suggests they are potentially interesting novel sources of antimicrobial metabolites.

Objectives

A set of 300 *Burkholderia* strains was selected from our in-house strain collection and screened for antimicrobial activity.

Methods

An agar overlay assay was used to screen for activity against *Staphylococcus aureus*, *Acinetobacter baumannii* and *Candida albicans.* A selection of strains showing antimicrobial activity in this first screen was subjected to a more in-depth analysis to determine the spectrum of activity against Gram-positive, Gram-negative and fungal pathogens. An additional panel of 40 recent clinical isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* was used to further explore the spectrum of activity against Gram-negative pathogens.

Conclusions

In the first screen for antimicrobial activity, 39% of the strains showed activity against one or more pathogens. Nineteen of these strains had activity against the Gram-negative pathogen *A. baumannii* and 28 strains were selected for the second screen. The large majority (n = 25) of these 28 strains had broad activity against Gram-positive pathogens and half of the strains showed activity against multiple drug-resistant Gram-negative pathogens, including *A. baumannii, Klebsiella pneumoniae, Enterobacter* spp. and *Escherichia coli*.

FEMS7-1369
Pathogens / Pathogenicity

ONE GENE AND TWO PROTEINS: A LEADERLESS MRNA SUPPORTS THE TRANSLATION OF A SHORTER FORM OF THE SHIGELLA VIRF REGULATOR.

M.L. Di Martino[1,2], C. Romilly[3], E.G.H. Wagner[3], B. Colonna[2], M.E. Sellin[1], G. Prosseda[2]
[1]Uppsala University,
Science for Life Laboratory- Department of Medical Biochemistry and Microbiology, Uppsala, Sweden
[2]Sapienza University of Rome, Istituto Pasteur Italia-
Fondazione Cenci Bolognetti- Department of Biology and Biotechnologies "C. Darwin", Rome, Italy
[3]Uppsala University, Department of Cell and Molecular Biology, Uppsala, Sweden

Backgrounds

VirF is an AraC-like regulator controlling virulence gene expression in *Shigella* spp., one of the main cause of bacillary dysentery in humans. VirF protein has been identified in different forms, but only the longer one, $VirF_{30}$, has been considered as an active form.

Objectives

This work investigates the function of $VirF_{21}$, a shorter form of the VirF regulator, its translation and its impact on *Shigella* virulence.

Methods

Site specific mutagenesis was performed to show the independent translation of VirF forms. Transcriptional *lacZ*-fusions and qRT-PCR were used to assess VirF forms functions. DNAseI footprinting was performed to map $VirF_{21}$ binding site on *virF* promoter. Transcriptional *lacZ*-fusions and Primer Extension were used to identify a new *virF* gene internal promoter. *In vivo* and *in vitro* translation as well as Toeprint Assays were performed to show that the newly identified *virF* leaderless mRNA is $VirF_{21}$ translational competent.

Conclusions

Here we show that in *Shigella*, the VirF protein is present in two different forms, independently translated. $VirF_{30}$ is responsible for activation of the virulence system, whereas $VirF_{21}$ negatively autoregulates *virF* expression itself. In addition we provide evidence supporting that $VirF_{30}$ and $VirF_{21}$ are translated by a single full length mRNA. $VirF_{21}$ can be also translated exclusively by a leaderless mRNA form, transcribed from a newly identified gene internal promoter. These new insights into the fine-tuned regulation of *Shigella* virulence open unexpected possibilities in the study of *Shigella* virulence and host cell invasion mechanisms.

FEMS7-2496
Pathogens / Pathogenicity

FUNCTIONAL ANALYSIS OF A TRIMERIC AUTOTRANSPORTER ADHESIN DETECTED IN AN AVIAN PATHOGENIC ESCHERICHIA COLI STRAIN

T. Rojas[1], L. da Silva[1], J. de Paiva[1], R. Maluta[1], J. Leite[1], R. Conceição[1], G. Nakazato[2], W. Dias da Silveira[1]
[1]University of Campinas, Genetics- Evolution and Bioagents, Campinas, Brazil
[2]Center of Biological Sciences- State University of Londrina, Department of Microbiology, Londrina, Brazil

Backgrounds

Autotransporters (AT) are proteins with the ability to pass through the outer membrane of Gram-negative bacteria via the type V pathway. Trimeric autotransporter adhesins (TAAs) are a subcategory of AT that form stable trimers on bacterial outer membranes. Using *in silico* analysis, we detected a 5253-bp region encoding a putative TAA in an Avian Pathogenic *Escherichia coli* (APEC) strain. This putative TAA is 80.6% similar to the UpaG TAA.

Objectives

We aimed to perform functional analysis of this protein in APEC strain SEPT362.

Methods

A comparative study with a wild type strain and null TAA mutant demonstrated that there was no statistical difference between both isolates regarding motility, biofilm production, lethality to day-old chicks, invasion to CEC32 and HEp-2 cells, survival in HD11 cells, and adhesion to Chicken Embryo Fibroblast (CEF) cells with mannose analogue. On the other hand, the null mutant presented a decreased ability to be internalized into HeLa cells ($p < 0.01$) and to adhere to CEF cells without a mannose analogue ($p < 0.01$). An enrichment analysis of the transcriptome of the wild type and the mutant indicated a downregulation of the regulator *mod*E. This regulator represses the molybdenum ABC transport system. The upregulation of this ABC transport system seems to have modified the expression of anaerobic metabolic pathways and some virulence factors, such as type 1 fimbria and flagella.

Conclusions

These results indicate that this TAA is associated with increased fitness to colonize anaerobic environments and decreased adherence mediated via type-1 fimbria.

FEMS7-1807
Pathogens / Pathogenicity

HAMAMELIS VIRGINIANA GLYCOLIC EXTRACT: IN VITRO STUDY OF ANTIMICROBIAL ACTIVITY AGAINST BACTERIAL AND FUNGAL BIOFILMS

L. DIAS OLIVEIRA[1], I. AMENDOLA[1], D. JESUS[2], F.E. OLIVEIRA[2], N. RONCONI COSTA[2], E. TAVARES FREITAS[2], J.R. OLIVEIRA[2], B.B. GRAZIELA[2], C. TALGE CARVALHO[3]
[1]São Paulo State University UNESP- Institute of Science and Technology, Biosciences and Oral Diagnosis, SÃO JOSÉ DOS CAMPOS, Brazil
[2]São Paulo State University UNESP- Institute of Science and Technology, Biosciences and Oral Diagnosis, SÃO JOSE DOS CAMPOS, Brazil
[3]São Paulo State University UNESP- Institute of Science and Technology, Restorative Dentistry, São Jose dos Campos, Brazil

Backgrounds

Taking into consideration the resistance of microorganisms to antibiotics, it becomes of great interest to analyze alternative antimicrobial agents, such as plant extracts.

Objectives

Evaluating *in vitro* antimicrobial activity of *Hamamelis virginiana* extract in contact with *Candida albicans* (CA), *Staphylococcus aureus* (SA), *Enterococcus faecalis* (EF) or *Streptococcus mutans* (SM) biofilms, during 5 min and 24 h.

Methods

The concentrations of 50, 100 and 200 mg/mL (n=10) were analyzed in both exposure times on each microbial 48 h-mature biofilm (n=10) in 96-wells microplates. Saline was used as control. After treatment, biofilm cells were scraped off and plated. After incubation (37°C/48 h), colony forming units per milliliter (CFU/mL) values were analyzed (ANOVA and Tukey test, 5%). There was a significant reduction of biofilms in comparison to the control group ($p<0.05$), in both exposure times. For EF, all the concentrations of extract showed CFU/mL reduction greater than 55% in the period of 5 min and greater than 90% in the period of 24 h. For SA, reduction was greater than 60% in 5 min and, in the period of 24 h, it was 97.97%. For SM, there was a reduction greater than 80% in 5 min and, in the period of 24 h, it was 92%. For CA, the extract promoted reduction of 80.90% in 5 min and, in 24 h, it promoted a reduction greater than 90%.

Conclusions

Hamamelis virginiana extract showed expressive antimicrobial action in contact with *S. mutans, S. aureus, E. faecalis* and *C. albicans* biofilms during 5 min and 24 h.

FEMS7-1944
Pathogens / Pathogenicity

ANTIMICROBIAL EFFECTS OF PFAFFIA PANICULATA AND JUGLANS REGIA EXTRACTS AGAINST CANDIDA ALBICANS, STREPTOCOCCUS MUTANS AND PSEUDOMONAS AERUGINOSA

L.D. OLIVEIRA[1], L.P. RAMOS[1], C.A. PEREIRA[1], J.R. OLIVEIRA[1], F.E. OLIVEIRA[1], G. BACK BRITO[1], C.A.T. CARVALHO[2]
[1]São Paulo State University UNESP- Institute of Science and Technology, Biosciences and Oral Diagnosis, São Jose dos Campos, Brazil
[2]São Paulo State University UNESP- Institute of Science and Technology, Restorative Dentistry, São Jose dos Campos, Brazil

Backgrounds

Popular use of plant extracts demonstrates antimicrobial activity, however, it is necessary scientific studies that prove this action aiming correct therapeutic indication.

Objectives

This study aimed to investigate the antimicrobial effects of *Pfaffia paniculata* and *Juglans regia* extracts on *Candida albicans* (CA), *Streptococcus mutans* (SM) and *Pseudomonas aeruginosa* (PA) planktonic cultures and biofilms.

Methods

ATCC strains of *C. albicans, S. mutans* and *P. aeruginosa* were used. Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (CMM) of extracts were determined by microdilution broth method. Mature biofilms with 48 h in 96-well plates were treated for 5 min with 200, 100 and 50 mg/mL of each extract and measured by violet crystal and MTT tests. Results were analyzed by ANOVA and Tukey test, 5%. Biomass of CA, SM and PA reduced, respectively, 45.6, 14.7 and 23.5% in contact with *P. paniculata* at 200 mg/mL. When in contact with *J. regia*, at 200 mg/mL, CA, SM and PA biomass reduced 31.0, 14.7 and 23.5%, respectively. MTT assay indicated reductions of 77.4% for CA, 20.3% for SM and 78.6% for PA with the use of *P. paniculata* extract and with *J. regia*, reductions were 61.0% for CA, 13.6% for SM and 26.8% for PA.

Conclusions

P. paniculata e *J. regia* extracts showed microbicidal activity against *C. albicans, S. mutans* and *P. aeruginosa* planktonic forms and biofilms.

FEMS7-0723
Pathogens / Pathogenicity

DRUG RESISTANT AND HOSPITAL SOURCES OF ASPERGILLUS CAUSING HOSPITAL ACQUIRED INFECTIONS

K. Diba[1], P. Badiee[2], A. Namaki[3]
[1]Urmia University of Medical Sciences, Medical Mycology, Urmia, Iran
[2]Shiraz University of Medical Sciences, Prof. Alborazi Microbiology Research Center, Shiraz, Iran
[3]Arefian General Hospital, Gynecology, Urmia, Iran

Backgrounds

In spite of a low percent (1%) of fungal hospital acquired infections, *Aspergillus* species are the main agents of fulminate fungal infections. Invasive aspergillosis has a mortality rate of 90%, among the *Aspergillus* infections.

Objectives

Regarding high frequently of *Aspergillus* spp. isolated from clinical and environmental sites in the nephrology ward, we tried to determine the azole resistance and perform a molecular epidemiologic study finding accurate and exact environmental sources for aspergillus infections and colonization in the large, general hospitals.

Methods

Our subjects included clinical specimens of case with HAI which collected during 48 month from October 2012 to September 2016 at the UMSU educational hospitals, Urmia, Iran. Also, environmental specimens including sterile swabs from surfaces of floor, walls, Curtains, beds, trolleys, air condition and cooling systems, medical devices were obtained as well as some samples from finger touches of the cast and visitors. The MICs for the azole family (fluconazole and ketoconazole) were described as the lowest concentration of the drug that could reduce 50% of fungal growth. The molecular method RAPD-PCR using six random Aspergillus primers was performed to study the hospital sources of the isolated Aspergillus.

Conclusions

Conclusions: The results of experimental studies on the specimens showed 93(47%) positive for a fungal or bacterial infections from the above case, 54(58%) had a fungal infection. Among the isolated *Aspergillus*, *A. flavus* (47%), *A. fumigatus* (29.4%) and *A.niger* (23.6%) were the most frequent. Using RAPD-PCR, two clinical-environmental sets including *A. niger* (sinus mass -floor) and *A. flavus* (BAL-air conditioner) were matched. All Aspergillus commonly clinical and environmental isolates were susceptible in MIC test. As expected, all three of the isolates obtained from one patient showed identical patterns (RAPD combined type A/D-12). However, the same pattern was found in two environmental isolates obtained from the wards.

FEMS7-0497
Pathogens / Pathogenicity

IDENTIFICATION OF CLINICAL VARIABLES AND COMMENSAL BACTERIAL SPECIES ASSOCIATED WITH INTESTINAL COLONIZATION BY MULTIDRUG-RESISTANT ENTEROBACTERIACEAE IN ACUTE LEUKEMIA PATIENTS

A. Djukovic[1], E. Gonzalez[2], J. Sanz[3], B. Herrera[1], J.L. Lopez Hontangas[2], M. Salavert[4], M.D. Gomez[2], M.A. Sanz[3], L. Debrauwer[5], B. Kuster[6], J.M. Rolain[7], K. Xavier[8], J. Xavier[9], C. Ubeda[1]
[1]Fundacion Para el fomento de la Investigacion Sanitaria y Biomedica de la Comuni, Genomica y Salud, Valencia, Spain
[2]Hospital Politecnico La Fe, Microbiologia, Valencia, Spain
[3]Hospital Politecnico La Fe, Hematologia, Valencia, Spain
[4]Hospital Politecnico La Fe, Enfermedades Infecciosas, Valencia, Spain
[5]INRA, Mass spectrometry, Toulouse, France
[6]Technische Universität München, Proteomics and Bioanalytics, Freising, Germany
[7]The Méditerranée Infection Foundation, Microbiology, Marseille, France
[8]Instituto Gulbenkian de Ciencia, Bactyerial Signaling Group, Oeiras, Portugal
[9]Memorial Sloan Kettering Cancer Center, Computational Biology, New York, USA

Backgrounds

Multidrug-resistant Enterobacteriaceae (MRE), including *Escherichia coli* and *Klebsiella pneumoniae*, are a major health problem to hospitalized patients. Infections with MRE frequently begin with the pathogen colonizing the intestine but, in normal conditions, the invader encounters a suppressive intestinal microbiota that resists colonization. Antibiotic treatments often disrupt the microbiota and accidentally open the way to MRE colonization. Despite its clinical relevance many human protective bacterial species and how they suppress MRE colonization remain unknown.

Objectives

The objective of our study is identifying commensal microbiota species and clinical variables that influence resistance to colonization by MRE.

Methods

We collected 817 fecal samples from 140 patients with acute leukemia, sampled weekly during hospitalization, over a period of eighteen months. Fecal samples were grown in selective media to determine MRE intestinal levels. We obtained the microbiota composition of each fecal sample by high-throughput sequencing of the 16s rRNA gene. The previously utilized Lotka-Volterra mathematical model (Buffie et al., Nature, 2015), was extended to identify both commensal bacteria and clinical variables that impact MRE intestinal colonization.

Conclusions

We determined that the type of antibiotic treatment received was among the strongest clinical variables influencing MRE intestinal levels. Interestingly, samples obtained during the transplant period show significantly lower MRE levels compared to those obtained during the chemotherapy. The Lotka-Volterra model identified four bacterial operational taxonomic units (OTUs) belonging to the genera *Lactobacillus*, *Bacteroides* and *Streptococcus* as those associated with decrease in MRE intestinal levels. These findings may lead to new probiotic therapies against MRE infection.

FEMS7-1194
Pathogens / Pathogenicity

VIRULENCE GENES, O-SEROGROUPS AND F18 VARIANTS OF PATHOGENIC ESCHERICHIA COLI FROM DIARRHEIC PIGLETS IN KOREA

K.H. Do[1], J.W. Byun[2], W.K. Lee[1]
[1]Chungbuk National University College of Veterinary Medicine, Veterinary Bacteriology, Cheongju, Republic of Korea
[2]Animal and Plant Quarantine Agency, Department of Animal and Plant health research, Gimcheon, Republic of Korea

Backgrounds

Escherichia (*E*.) *coli* have been divided into several pathotypes according to their combinations of virulence factors. Among them, Enterotoxigenic *E. coli* (ETEC) and shiga toxin-producing *E. coli* (STEC) produce fimbriae and toxins, and cause diarrhea and edema disease in pigs. Three F18 antigenic variants (F18ab, F18ac and F18new) are frequently associated with ETEC or STEC.

Objectives

We examined prevalence of the O-serogroups and virulence genes among *E. coli* strains and investigated the frequency of antigenic variants in F18 positive ETEC and STEC isolated from diarrheic piglets in Korea.

Methods

Between 2007 and 2016, a total of 862 *E. coli* isolated from diarrheic piglets were serogrouped and tested for the fimbrial adhesin and toxin genes. The genes for the toxins and adhesins were amplified by PCR. Among them, 260 F18 positive isolates were differentiated F18 antigenic variants using real-time PCR technique.

Conclusions

In this study, the predominant serogroup was O139, and the most frequently detected adhesin was F18. Among 323 F18-encoding isolates, 111 (ETEC/STEC) and 90 (STEC) isolates were positive for Stx2e gene. We tested 260 F18-encoding isolates to discriminate F18 subtypes, and F18ac (145 isolates) was the most predominant F18 subtype. There were 58 (ETEC/STEC) and 8 (STEC) isolates which are positive for Stx2e gene. These indicate that there is high risk for edema disease to diarrheic piglets in Korea. This results provide not only epidemiological data regarding the prevalence of serogroups and virulence factors but could also be used to design control measures for colibacillosis in Korean piggeries.

FEMS7-2554
Pathogens / Pathogenicity

COMPARATIVE ANALYSIS OF ESCHERICHIA COLI ISOLATES FROM BOVINE ORIGIN: IS THERE A SPECIFIC MASTITIS PATHOTYPE?

A. Leimbach[1], A. Poehlein[2], R. Daniel[2], U. Dobrindt[1]
[1]University of Münster, Institute of Hygiene, Münster, Germany
[2]University of Göttingen, Göttingen Genomics Laboratory, Göttingen, Germany

Backgrounds

Escherichia coli is a major pathogen of acute bovine mastitis. Pathogenic *E. coli* are traditionally classified into different pathotypes based on specific virulence factor sets and characteristic disease pathology. Although a mammary pathogenic *E. coli* pathotype has been proposed, no common virulence factors could be determined despite extensive research efforts.

Objectives

Environmental factors as well as the bovine innate immune response have been implicated as the sole parameters influencing mastitis outcome. Nevertheless, many studies tried to detect specific virulence factors subsets in *E. coli* mastitis isolates, with no success. Thus, we followed a whole genome and phenotypic approach to identify putative virulence traits by comparing bovine mastitis and commensal *E. coli* isolates.

Methods

A comparative genomics approach was used to characterize the gene content and phenotype of the strains in relation to phylogeny and pathogenicity.

Conclusions

Mastitis-associated *E.coli* (MAEC) and commensal strains could not be unambiguously discriminated by their phylogenetic background and the presence of virulence-associated genes. Nevertheless, a certain correlation between MAEC and phylogroup A and commensal isolates and phylogroup B1 has been observed. Gene content is significantly affected by phylogenetic background. MAEC mirror the phylogenetic and genotypic diversity of isolates from the gastrointestinal tract. There is no evidence for a mammary gland-specific pathotype. Putative virulence factors should rather be considered fitness factors for gastrointestinal colonization. MAEC are facultative pathogens that are recruited from the normal intestinal microbiota.

FEMS7-3057
Pathogens / Pathogenicity

COMPARTIVE GENOME-WIDE TRANSCRIPTIONAL ANALYSIS OF TWO CANDIDA ALBICANS MUTANTS AFFECTED IN N-GLYCOSYLATION

A. Dominguez[1], *C. Cívicos*[1], *M.C. López*[1]
[1]*Universidad de Salamanca, Microbiology and Geneticas, Salamanca, Spain*

Backgrounds

Candida albicans is one of the most important opportunistic human pathogens, causing life-threatening infections in immunocompromised individuals. Protein glycosylation is important for folding, regulated secretion, localization and function of proteins. Defects in protein glycosylation cause surface alterations of cell wall, the first point of contact to host cell, therefore affecting pathogenicity attributes like adhesion and morphological plasticity that allow *Candida* to adapt to different ecological niches of the host. We constructed two *C. albicans* mutants, *mnn1Δ/Δ* and *mnn9 Δ/Δ*, which are defective in a α-1,3 and α-1,6 mannosyltransferases, respectively. We analyzed their expression profiles generated under different growth conditions with the aim of knowing the cell responses to glycosylation defects

Objectives

In order to investigate the importance of N-glycan modifications of proteins in morphogenetic and pathogenic processes in *C. albicans*, we have performed global expression analysis of mutants *mnn9Δ/Δ* and *mnn1Δ/Δ* affected in the N-glycosylation pathway in the Golgi Complex

Methods

C. albicans mutants have been constructed using the “Ura-blaster” method. Transcriptional profiling was carried out using cDNA microarrays purchased from Eurogentec (Seraing, Belgium). The data analysed included 4 replicates.

Conclusions

The *mnn9Δ/Δ* transcriptional profiles are consistent with their phenotypic characteristics, such as cell aggregation and inability to form true hyphae. Regarding these phenotypes, we did not detect any difference between *mnn1Δ/Δ* and the parental strain, the transcriptome of this mutant being in accordance with these results. Here we present other transcriptional features regarding to alterations in signalling pathways in response to glycosylation defects

FEMS7-0508
Pathogens / Pathogenicity

INACTIVATION OF THE PST TRANSPORTER DEREGULATES THE C-DI-GMP PATHWAY AND DECREASES EXPRESSION OF TYPE 1 FIMBRIAE BY UROPATHOGENIC ESCHERICHIA COLI STRAIN CFT073

S. CRÉPIN[1], G. PORCHERON[1], S. HOULE[1], J. HAREL[2], C. MARTIN[3], C.M. DOZOIS[1]
[1]INRS-Institut Armand-Frappier, INRS-Institut Armand-Frappier, Laval- Quebec, Canada
[2]Université de Montréal, CRIPA and Faculté de médecine vétérinaire, Saint-Hyacinthe- Québec, Canada
[3]Institut National de la Recherche Agronomique, Laboratoire de Microbiologie, Saint-Genès-Champanelle, France

Backgrounds

The *pst* system encodes the phosphate transporter (Pst). Inactivation of Pst constitutively activates the two-component regulator PhoBR and has been shown to attenuate virulence of a variety of bacterial pathogens. In uropathogenic *Escherichia coli* strain CFT073, attenuation by inactivation of *pst* is predominantly attributed to the decreased expression of type 1 fimbriae.

Objectives

To elucidate mechanisms connecting the Pst system and decreased expression of type 1 fimbriae.

Methods

A transposon library in the *pst* mutant was generated and clones were tested for a regain in type 1 fimbriae production. The diguanylate cyclase encoded by *yaiC* (*adrA*) was identified to link the Pst system and type 1 fimbrial expression. In the *pst* mutant, *yaiC* was induced and shown to contribute to decreased expression of type 1 fimbriae by predominantly altering expression of the FimBE-like recombinases IpuA and IpbA, affecting at the same time, orientation of the the *fim* invertible promoter. In the *pst* mutant, inactivation of *yaiC* restored *fim*-mediated adhesion to epithelial cells and colonization of mouse bladders and kidneys. Expression of *yaiC* required PhoB, and transcription of *yaiC* was linked to the PhoB-dependent *phoA-psiF* operon. As YaiC is involved in c-di-GMP biosynthesis, an increased accumulation of c-di-GMP was observed in the *pst* mutant.

Conclusions

Overall, results suggest that one mechanism by which inactivation of the Pst system reduces expression of type 1 fimbriae is through PhoBR-mediated activation of *yaiC*, which in turn increases accumulation of c-di-GMP, represses the *fim* operon and consequently, attenuates virulence in the mouse urinary tract infection model.

FEMS7-2984
Pathogens / Pathogenicity

DEVELOPMENT OF POLYMER-IMPREGNATED SILICONE URINARY CATHETERS RESISTANT TO PROTEUS MIRABILIS SURFACE ADHESION AND SWARMING MOTILITY

J.F. Dubern[1], A.L. Hook[2], A. Carabelli[2], J. Luckett[1], C.Y. Chang[3], P.E. Magennis[2], M.R. Alexander[2], P. Williams[1]
[1]Centre for Biomolecular Sciences, School of Life Sciences, Nottingham, United Kingdom
[2]Laboratory of Biophysics and Surface Analysis, School of Pharmacy, Nottingham, United Kingdom
[3]School of Life Sciences, Heriot-Watt University, Edinburgh, United Kingdom

Backgrounds

Proteus mirabilis is an opportunistic pathogen that causes complicated urinary tract infections (UTIs). *P. mirabilis* cells utilize the swarming behaviour to rapidly migrate across catheters and ascent the urinary tract. *P. mirabilis* employs a wide variety of virulence factors during the initial colonisation. One major virulence factor is urease, an enzyme that hydrolyses urea to free ammonia resulting in subsequent precipitation of calcium and magnesium phosphate. The alkaline condition leads to the formation of kidney stones and the crystalline biofilms that encrust the catheter tubing.

Objectives

Through structure-activity analysis, we investigate the ability of homopolymers to inhibit surface attachment and motility of *P. mirabilis* and other pathogens in order to identify pan inhibitory materials.

Methods

Several anti-attachment materials formed from acrylates with hydrocarbon pendant groups have been developed.Their ability to resist attachment of fluorescently labelled *P. mirabilis* to urinary catheters and to prevent cell migration was investigated. In addition, *P. mirabilis* swarming behaviour was examined using Differential Interference Contrast microscopy (DIC) and scanning electron microscopy.

Conclusions

In this study a new monomer combination formed from acrylates with hydrocarbon pendant groups is presented that is able to resist both the adhesion and swarming of *P. mirabilis.* The new coating is non-toxic and prevents attachment of other pathogens including *Pseudomonas aeruginosa*, *Staphylococcus aureus* and uropathogenic *Escherichia coli.* In addition, the microscopic examination of *P. mirabilis* swarming behaviour suggests its ability to sense and respond to surfaces of different chemical composition *via* an unknown molecular mechanism.

FEMS7-2988
Pathogens / Pathogenicity

SUPPRESSIVE SOILS AGAINST GAEUMANNONYCES GRAMINIS OF SOILS UNDER MONOCULTURE MANAGEMENT IS REGULATED BY RHIZOSPHERE MICROORGANISMS

P. Duran[1], *M.D.L.L. Mora*[1]
[1]*Universidad de La Frontera, Scientific and Technologic Bioresorce Nucleus, Temuco, Chile*

Backgrounds

Wheat production around the world is severely compromised by the occurrence of “take-all” disease, which is caused by the soil-borne pathogen *Gaeumannomyces graminis* var tritici (Ggt). In this context, suppressive soils are those environments where plants comparatively suffer less soil-borne pathogen diseases than expected owing to the activities of native soil microorganisms.

Objectives

this study aimed to screen the occurrence of Ggt-suppressive soils from southern Chile and we compared the structure of the communities in suppressive and conductive soil

Methods

Two preliminary screenings for soils inhibition of Ggt growth *in vitro* allowed the identification of nine putative suppressive soils, and 7 of them were confirmed to be Ggt-suppressive in plant assays under greenhouse conditions. Supressiveness was lost upon sterilization of the soils, and recovered by adding 1% of non sterilized soil. The structure of the community was studied in both suppressive and conducive soils

Conclusions

Soil suppression was associated to the soil microbiota. Remarkably, bacteria community composition was similar in suppressive soils when compared with that in positive disease conducive control soil. Accordingly, suppressive soils constitute an important resource for the development of environmentally friendly and efficient biotechnological applications for the control of soil-borne diseases.

This study was supported by FONDECYT Iniciation Project No. 11150540

FEMS7-0878
Pathogens / Pathogenicity

ST131 ESCHERICHIA COLI AND ITS H30 AND H30-RX SUBCLONES IN TURKEY

D. Er[1], D. Dundar[2], H. Uzuner[1]
[1]Kocaeli University- Institute of Health Sciences, Medical Microbiology, Kocaeli, Turkey
[2]Kocaeli University- Faculty of Medicine, Medical Microbiology, Kocaeli, Turkey

Backgrounds

ST131 *Escherichia coli* (*E. coli*) is one of the leading causative agent of urinary tract infections worldwide with mutidrug-resistance. The *fimH* alleles H30 and H30-Rx are associated with infections of ST131 *E. coli*.

Objectives

The ST131 *E. coli* has been identified in only few studies from our region and the prevalence of subgroups are not well known. The aim of this study is to assess *E. coli* ST131 and its subclones H30 and H30-Rx in uropathogenic *E. coli* isolates.

Methods

A total of 250 uropathogenic *E. coli* isolates were included in this study. Clinical features including age, sex, complication (complicated-uncomplicated), syndrome (cystitis-pyelonephritis), origin (community acquired-healthcare associated) were obtained. Phylogenetic determination was identified with multiplex PCR reaction. O25b, O16 and SNP PCRs were applied for isolates which belong to B2 phylogroup for ST131 determination. Antimicrobial resistance was determined by disk diffusion test for 16 antimicrobial agents. Also, ST131-positive isolates were investigated for H30, H30-Rx subclones and 8 adhesin virulence genes including *fimH*, *iha*, *papAH*, *sfa/focDE*, *afa/draBC*, *focG*, *gafD* and *bmaE*.

Conclusions

There is not enough information about the frequency of ST131 *E. coli* and its H30, H30-Rx subclones in Turkey. In this study, prevalence of ST131 isolates for this collection was 15,6%. ST131 isolates usually carry *fimH*, *iha* and afa/*draBC* genes; also ESBL and MDR positive. Investigation of the relationships between adhesin virulence profiles, clonal groups and host factors will provide the understanding of the pathogenesis. The determination of these relations may result in alternative treatment approaches geared towards virulence properties.

FEMS7-1195
Pathogens / Pathogenicity

BIOFILM FORMATION AND VIRULENCE GENES AMONG MULTIDRUG RESISTANT A.BAUMANNII STRAINS ISOLATED FROM INTENSIVE CARE UNITS

Ö. Altınok Özgün[1], B. Boral[2], M.A. Ergin[3], Ö. Köseoglu Eser[4]
[1]İstanbul Aydın Unversity, Department of Medical Microbiology, İstanbul, Turkey
[2]Çukurova University, Department of Medical Microbiology, Adana, Turkey
[3]Hacettepe University, School of Health Services- Medical Laboratory Programme, Ankara, Turkey
[4]Hacettepe University, Department of Medical Microbiology, Ankara, Turkey

Backgrounds: Multidrug resistant Acinetobacter baumannii isolates lead to epidemics and loss of patients in the hospital setting.
Objectives: The aim of this study was to determine the relationship between the existence of biofilm and virulence genes among 156 invasive MDR A.baumannii isolates.
Methods: Isolates with known antibiotic susceptibility and PFGE patterns from ten ICUs of medical centers in five different geographical region of Turkey were included in the study. Quantitative microplate biofilm method was used to detect biofilm production. Formation of biofilm was interpreted as weak, moderate and strong. The presence of virulence genes bap, csgA, csuE, fimH, ompA and blaper-1 was investigated by PCR. A.baumannii ATCC 19606 was used as positive control.
Conclusions: Among all isolates, 60.25% (94/156) showed biofilm formation ability. Of the ninety four isolates, 17 were weakly, 33 were moderately and 44 were strongly positive. The mean biofilm biomass formation capacity of the negative/positive controls and isolates obtained during the test procedures was 2.2371±0.0033. Biofilm producing isolates were most frequently in pulsotype II 19.14%(18/94) and pulsotype IX 17.02%(16/94) and 26.59%(25/94) of the biofilm producer isolates were from a single center in PFGE profile. The frequency of genes bap, csgA, csuE, fimH, ompA and blaper-1 among the isolated strains (n:156) were 44.23%, 70.51%, 32.05%, 7.05%, 21.79% and 3.2%, respectively. This study shows that there is a strong relationship between the resistance and biofilm formation in invasive A.baumannii isolates and the patients with MDR invasive A.baumannii isolates should be evaluated in conjunction with the virulence factors and clonal spread.

FEMS7-0940
Pathogens / Pathogenicity

DIIA IS A CELL WALL PROTEIN OF STREPTOCOCCUS PNEUMONIAE INVOLVED IN INVASIVE DISEASE THAT SHOWS POTENTIAL AS VACCINE CANDIDATE

M.S. Escolano-Martínez[1], A. Domenech[2], J. Yuste[3], M.I. Cercenado[3], C. Ardanuy[4], J. Liñares[4], A. G. de la Campa[5], A.J. Martin-Galiano[3]
[1]Instituto de Salud Carlos III. Centro Nacional de Microbiología, Laboratorio de referencia e investigación en enferemdades bacterianas prevenibles por vacunas, Madrid, Spain
[2]University of Groningen, Microbiology, Groninga, The Netherlands
[3]Instituto de Salud Carlos III. Centro Nacional de Microbiología, Laboratorio de referencia e investigación en enfermedades bacterianas prevenibles por vacunas, Madrid, Spain
[4]Hospital de Bellvitge, Servicio de Microbiología, Barcelona, Spain
[5]Instituto de Salud Carlos III. Centro Nacional de Microbiolgía, Laboratorio de referencia e investigación en enfermedades bacterianas prevenibles por vacunas, Madrid, Spain

Backgrounds

Many surface proteins show allelic variants with different number of repeats which affects to their function and immunogenicity.

Objectives

- To analyze the outer protein DiiA (Dimorphic invasion-involved protein A) encoded by the *sp1992* locus of *Streptococcus pneumoniae* TIGR4: specificity, epidemiology, role in pathogenesis and biological function.

- To study the contribution of different regions of protein DiiA to vaccine efficacy in terms of immunological response and level of protection.

Methods

DiiA architecture was determined using bioinformatic algorithms. The occurrence of *diiA* alleles was screened in clinical isolates by PCR. Isogenic mutants were constructed to study the relevance of DiiA sections in murine models. DiiA binding to human molecules was determined by surface plasmon resonance. Immunization assays were carried out in murine models using different purified DiiA variants. Immunoglobulin production was quantified by ELISA. Protection against pneumococcal sepsis and carriage challenges was quantified in mice through survival rate and bacterial load.

Conclusions

DiiA has a classical adhesin topology. This protein is involved in processes of colonization, pneumonia and sepsis, probably through the interaction to lactoferrin and collagen. DiiA is a promising vaccine candidate against *Streptococcus pneumoniae*.

FEMS7-1052
Pathogens / Pathogenicity

MULTIDRUG RESISTANT TUBERCULOSIS IN ETHIOPIAN SETTINGS AND ITS ASSOCIATION WITH PREVIOUS HISTORY OF ANTI-TUBERCULOSIS TREATMENT: A SYSTEMATIC REVIEW AND META-ANALYSIS

S. Eshetie[1], M. Gizachew[1], M. Dagnew[1], H. Woldie[2], B. Tessema[1], F. Moges[1]
[1]University of Gondar-College of Medicine and Health Sciences, Medical Microbiology, Gondar, Ethiopia
[2]University of Gondar-College of Medicine and Health Sciences, Department of Nutrition, Gondar, Ethiopia

Backgrounds

Multi-drug resistant *tuberculosis* (MDR-TB) is remains a major public health concern in Ethiopia. Little is known about the burden of disease at national level.

Objectives

This meta-analysis is aimed, firstly, to determine the pooled prevalence of MDR-TB among newly diagnosed and previously treated TB cases, secondly, to measure the association between MDR-TB with the history of previous anti-TB drugs treatment.

Methods

PubMed, Embase and Google Scholar databases were consulted. Studies that reported prevalence of MDR-TB among new and previously treated TB patients were selected. Forest plots of all prevalence estimates were performed and summary estimates were also calculated using random effects models. Associations between previous TB treatment and MDR-TB infection were examined through subgroup analyses stratified by new and previously treated patients.

We identified 16 suitable studies and found an overall prevalence of MDR-TB among newly diagnosed and previously treated TB patients, 1.7% (95% CI 1.2% - 2.3%) and 14.1% (95% CI 10.9% - 17.2%), respectively. The observed difference was statistically significant (P<0.01). For the past 10 years (2006 to 2014) the overall MDR-TB prevalence showed a stable time trend. There was an odds ratio of 8.1 (95% CI 7.5 – 8.7) for previously treated TB patients to develop a MDR-TB infection compared to newly diagnosed cases.

Conclusions

The burden of MDR-TB remains high, especially in previously treated TB cases. Previous TB treatment was the most powerful predictor for MDR-MTB infection. Therefore, strict compliance with anti-TB regimens and improving case detection rate are the necessary steps to tackle the problem in Ethiopia.

FEMS7-2132
Pathogens / Pathogenicity

PSEUDOMONAS AERUGINOSA CLINICAL ISOLATES: A RIBOSOMAL MUTATION CAUSES HIGH AMINOGLYCOSIDE RESISTANCE

R. Espinosa Portero[1], D. Matzov[2], A. Jiménez-Fernández[1], E. Zimmerman[2], A. Bashan[2], A. Yonath[2], H.K. Johansen[1], S. Molin[1]
[1]Technical University of Denmark, Biosustain, Lyngby, Denmark
[2]The Weizmann Institute of Science, Department of Structural Biology, Rehovot, Israel

Backgrounds

Pseudomonas aeruginosa is an opportunistic pathogen that causes chronic lung infections in Cystic Fibrosis (CF) patients. Clinical strains have been isolated over the years from sputum samples for genome sequencing. Bacterial ribosomes are formed by a small (30S) and a large subunit (50S). A mutation in the gene coding for 50S ribosomal protein L6 (*rplF*) has been recently described in two in *P. aeruginosa* clinical isolates from CF patients. These two mutants with a deletion in *rplF* gene have increased aminoglycoside resistance (to both gentamicin and tobramycin). Ribosomal proteins have a structural role in the organization of bacterial ribosomes, and protein L6 is located in the surface of the 50S subunit. Aminoglycosides have been described to interact with both subunits from ribosomes for translation inhibition

Objectives

To test whether gentamicin is able to bind to ribosomes with mutated L6 protein, and if this represents a growth advantage for these mutants. To determine what is causing the increase in aminoglycoside resistance. To determine translational fidelity

Methods

- Real-time PCR to assess the expression of ribosomal proteins
- Proteomic analysis to assess ribosomal protein abundance and screen effects on the mutation in the whole proteome
- *In vitro* translation to assess the role of gentamicin binding
- Ribosome quantification
- Fidelity assays

Conclusions

Final conclusions cannot be drawn currently, as this research is still going. Preliminary results suggest that ribosomal protein expression and abundance is not affected by *rplF* mutations. Results also show that gentamicin does not contribute to increased growth rate of *rplF* mutants.

FEMS7-2420
Pathogens / Pathogenicity

SUPERFICIAL MYCOSIS IN CHILDREN FROM PUEBLA MEXICO

A.P. Espinosa Texis[1], *E.M. Vidal López*[2], *D. Castillo Hernández*[3]
[1]*Benemerita universidad Autonoma de Puebla, ICUAP, Puebla, Mexico*
[2]*Universidad de Sonora, Departamento de Ciencias Quimico Biologicas, Sonora, Mexico*
[3]*Centro de Investigaciones en Biotecnología Aplicada IPN, Microbiología, Tlaxcala, Mexico*

Backgrounds

Within the superficial mycoses that occur in humans, ringworm and white piedra have been described in the infantile stage, the first being the most frequent. The white piedra is associated with diseases that cause immunosuppression as HIV/AIDS among others.

Objectives

The objective was determine the presence of cases clinical of ringworm and white piedra in children of the city of Puebla.

Methods

We´re taken 50 Hair samples in children with clinical lesions compatible with tinea capitis or white piedra. Five were positive to tinea capitis and two was positive for white piedra. The mycological diagnosis was performed by microscopic examination of hair and cultivation of the etiologic agent in agar glucose Sabouraud with and without antibiotics, incubating at 28° C for 15 days. The identification was carried out considering the morphology macroscopic and microscopic of the fungus.

The observation microscopic of the hair to them cases of ringworm showed arthoconidia type endothrix and ectothrix, for the cases of white piedra is observed *structures type ectothrix with filaments and arthoconidia rectangular, ovoid willing in groups.* The culture allowed isolate *Microsporum canis, Trichophyton rubrum*.and *Trichosporon.* Treatment included benzoate benzyl, oral itraconazole and ketoconazole topical.**Conclusions**

The Mycosis surface more frequent in children was the ringworm capitis, followed of the white piedra. Of the antifungal agents used the ketoconazole presented the best results.

FEMS7-2750
Pathogens / Pathogenicity

ADHESION OF PATHOGEN BACTERIA TO POLYSTYRENE, SKIN AND GUT MUCUS OF GILTHEAD SEABREAM (SPARUS AURATA), INFECTIOUS CAPACITY ON SAF-1 CELL LINE AND RESISTANCE TO ANTIBIOTICS

D. Ceballos-Francisco[1], B.H. Said[2], G. Francisco[1], C. Alberto[1], M.A. Esteban[1]
[1]University of Murcia, Cell Biology & Histology, Murcia, Spain
[2]University of Gabès, LaLaboratory of Microbiology, Gabès, Tunisia

Backgrounds

Giving increasing worldwide demand of seafood, there is an escalating need for research to identify solutions to fish health problems without any offensive uses of antibiotics.

Objectives

The capacity of ten pathogenic bacteria isolated from fish and marine environment, to adhere to polystyrene, skin mucus and gut mucus of gilthead seabream (*Sparus aurata*) was investigated. The pathogenicity of these strains was tested *in vitro* on gilthead seabream fibroblast cell line SAF-1. Additionally, the sensitivity of the isolates was tested for thirteen antibiotics having different mode of action.

Methods

Vibrio harveyi H28 (HQ449775) and *Halomonas venusta* (HQ727663) displayed a strong adhesion to polystyrene surface. *Dietzia maris* (HQ425656) has showed a strong adhesion to skin mucus substratum. Around 40% of isolates were moderately adherents. Interestingly, for gut mucus, there were no strongly adherents bacteria. For infectivity test, *D. maris* (HQ425656), *V. harveyi* H28 (HQ449775) and *Aeromonas bivalvium* (DQ504430) decreased the SAF-1 cells viability to 89% after only 3 hours of exposure. All isolates presented resistance to sulfonamide and 60% were resistant to both sulfonamide and penicillin G.

Conclusions

Present findings could be relevant in fish aquaculture and underscore the importance of the linkage between adhesion capacity, *in vitro* infectivity, and antibiotic susceptibility of pathogen bacteria to avoid fish diseases that causes serious economic lost in aquaculture.

Acknowledgements

F.A. Guardiola wants to thank the *Fundação para a Ciência e Tecnologia* (Portugal) for grant (SFRH/BPD/104497/2014). This research was funded by MINECO cofunded with European Regional Development Funds (ERDF/FEDER) (grant number AGL2014-51839-C5-1-R) and by the *Fundación Séneca de la Región de Murcia* (grant number 19883/GERM/15, *Grupo de Excelencia*).

FEMS7-1394
Pathogens / Pathogenicity

IDENTIFICATION OF BACTERIAL STRAINS FROM GUT MICROBIOTA ASSOCIATES WITH DIARRHEOGENIC E. COLI INFECTION

F. Mauricio[1], I. Mariana[1], G. Pablo[1], R.M. Ramon[2], V. Roberto[3]
[1]Centro de Estudios Moleculares,
Departamento de Pediatría- Hospital Luis Calvo Mackenna- Universidad de Chile, Santiago, Chile
[2]IMEDEA, Departament de Ecologia i Recursos Marins, Illes Balears, Spain
[3]ICBM, Facultad de Medicina- Universidad de Chile, Santiago, Chile

Backgrounds

Diarrheagenic *Escherichia coli* (DEC) strains remain a major cause of diarrhea in children under 5 years of age. DEC pathogenesis depends on the interaction of the pathogen with environmental factors such as the intestinal microbiota. Several studies have shown changes in the composition of the microbiota in diarrheal infections and that strains of the intestinal microbiota may participate in the pathogenic process of DEC strains. However, there is no much information of specific microbiota strains that might be involved in DEC infection.

Objectives

To identify cultivable strains of intestinal microbiota associated with DEC infections in children under 5 years of age

Methods

120 stool samples from children between 1 and 5 years of age analyzed by FilmArray GI® test were grouped whether they were positive for one of the DEC pathotypes, an enteric virus or a bacterial pathogen other than DEC; stool samples negative for pathogens and stool samples from healthy children negative for pathogens were included. In all samples, 16S ribosomal gene was sequenced to identify operative transcriptional units (OTUs) associated with each ethiological group. These OTUs were compared to a database of cultivable bacterial species, to determine the major species of the microbiota associated with each group.

Conclusions

We found variations in the microbiota in the different groups analyzed. In DEC group, strains belonging to the phylum *Firmicutes*, *Preoteobacteria* and *Bacteroidetes* were identified, the latter two being predominant in relation to the rest of the groups. Strains of *Bacteroides*, *Parabacteroides*, *Ruminococcus* and *Veilonella* present in greater proportion in the DEC group.

FEMS7-1336
Pathogens / Pathogenicity

SCREENING OF ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA LACTOBACILLUS SPP. AGAINST DRUG-RESISTANT STRAINS OF KLEBSIELLA SPP.

T. Fedorova[1], I.V. Rozhkova[2], E.Y. Agarkova[3], A.V. Begunova[4], K.A. Ryazantseva[3], A.G. Kruchinin[3]
[1]Federal State Institution "Federal Research Centre "Fundamentals of Biotechnology" of Russian Aca demy of Sciences, Bach Institute of biochemistry, Moscow, Russia
[2]Federal State Budgetary Scientific Institution "All-Russian Research Institute of Dairy Industry, Microbiology, Moscow, Russia
[3]Federal State Budgetary Scientific Institution "All-Russian Research Institute of Dairy Industry", Milk protein concentrates laboratory, Moscow, Russia
[4]Federal State Budgetary Scientific Institution "All-Russian Research Institute of Dairy Industry", Microbiology, Moscow, Russia

Backgrounds

A lot of *Lactobacillus* strains show antagonistic activity against the majority of pathogenic microorganisms including gram-negative bacteria which establish the probability of cross infective episode. *Klebsiella* spp. strains are the most dangerous among them. They often posses multiple antibiotic resistance and herewith resistance determinants circulate among different strains.

Objectives

The aim of the present study is screening of antagonistic activity of lactic acid bacteria strains collection against drug-resistance strains of *Klebsiella* spp. isolated as the source of hospital infection.

Methods

Lactobacillus spp. strains were identified by genetic-molecular method using the analysis of nucleotide sequence of 16S rRNA gene. The method of growing mixed populations in comparison with test strains growth in monoculture was used for determination of lactic acid bacteria antagonistic activity. Cultivation of strains was carried out on MRS medium, thermostating at 37C within 24-48 hrs.

Conclusions

The apparent antagonistic activity against hospital *Klebsiella* spp. strains of three lactic acid bacteria - *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Lactobacillus acidophilus* - has been proved. Within the first day of joint cultivation with *L. acidophilus*, *L. rhamnosus* and *L. reuteri* inhibition of the studied *Klebsiella* spp. strains compounded 13-28%, 36% and 60%, respectively; within the second day – 57-91%, 76-83% and up to 72%, respectively. Thus the discovered lactic acid bacteria strains can be used for development of antibiotic preparations and medical foodstuffs and for maintenance of patients health staying in hospitals especially on the background of immunosuppressive and radiation therapy.

This work was supported by Grant of Russian Science Foundation #16-16-00094

FEMS7-2706
Pathogens / Pathogenicity

CHARACTERIZATION OF HUMORAL AND CELLULAR IMMUNE RESPONSES TO YERSINIA PESTIS PLA ANTIGEN IN HUMANS IMMUNISED WITH LIVE PLAGUE VACCINE (LPV)

V. Feodorova[1,2], M. Khizhnyakova[1], A. Lyapina[1], S. Zaitsev[1], M. Telepnev[3], L. Sayapina[4], O. Ulianova[1,5], S. Ulyanov[1,6], T. Arseneva[7], I. Morozova[7], A. Trukhachev[7], S. Lebedeva[7], E. Lyapina[8], V. Motin[3]
[1]Saratov Scientific and Research Veterinary Institute- Federal Research Center for Virology and Micro biology- Branch in Saratov, Department for Zoo and Zooanthroponotic Bacterial Infections, Saratov, Russia
[2]Saratov State Agrarian University named after N.I. Vavilov, Department for Microbiology- Biotechnology and Chemistry, Saratov, Russia
[3]University of Texas Medical Branch, Departments of Pathology and Microbiology, Galveston- TX, USA
[4]Scientific Center on Expertise of Medical Application Products, Department of Vaccine Control, Moscow, Russia
[5]Saratov State National University, Scientific Bacteriological Laboratory, Saratov, Russia
[6]Saratov State National University, Department for Nano- and Biotechnology, Saratov, Russia
[7]Antiplague Research Institute, Laboratory of Microbiology of Yersinia pestis, Rostov-on-Don, Russia
[8]Saratov State Medical University named after V.I. Razumovsky, Department for Infectious Diseases, Saratov, Russia

Backgrounds

Y. pestis, a gram-negative bacterium causing plague, a deadly infectious disease. The outer membrane Pla protease is considered to be the virulence-associated multifunctional protein playing a critical role in pathogenesis of bubonic and pneumonic plague and providing detectable levels of the relevant antibodies (anti-Pla-Ab) in convalescent sera of human patients that survived plague infection.

Objectives

The goal of this study was to evaluate both human humoral and cellular immune responses to Pla antigen followed by mapping of linear immune-reactive epitope(s).

Methods

Sera from donors (n=34) with multiple immunizations with LPV, as well as from healthy non-immune controls (n=17), were analysed in ELISA for the presence of anti-Pla-Ab using highly purified recombinant Pla antigen. A lymphocyte proliferation assay and cytokine profile (IFN-gamma/TNF-alpha/IL-4/IL-10/IL-17A) in response to *in vitro* stimulation with recombinant Pla were also conducted. B-cell immune-reactive epitope mapping of the target antigen was performed in ELISA by using a library of 59 overlapping synthetic peptides (GenScript, USA).

Conclusions

We found that fifteen out of 34 (44.1%) sera samples from vaccinees and seventeen out 17 (100%) derived from naïve donors were positive for the presence of anti-Pla-Abs (titers ranged from 1:50-1:800) demonstrated the marked cross-reactivity of the Pla antigen. *In vitro*-proliferative response to Pla revealed a negligible production of IFN-gamma/TNF-alpha/IL-10. In vaccinees Pla reduced of IL-4 by 3.4-fold, but increased of IL-17A by 14.7-fold pointing to a possible Th17-polarisation of Pla-related immune response. Nine potential immune-reactive 15-mer peptides were identified by library screening.

Grant RFBS-No.-2616-34-00051

FEMS7-2707
Pathogens / Pathogenicity

DETECTION OF CHLAMYDIA TRACHOMATIS IN ABORTED CATTLE

V. Feodorova[1,2], *Y. Saltykov*[1,2], *T. Polyanina*[1], *S. Zaitsev*[1], *O. Ulianova*[1,3]
[1]*Saratov Scientific and Research Veterinary Institute- Federal Research Center for Virology and Micro biology- Branch in Saratov, Department for Zoo and Zooanthroponotic Bacterial Infections, Saratov, Russia*
[2]*Saratov State Agrarian University named after N.I. Vavilov,*
Department for Microbiology- Biotechnology and Chemistry, Saratov, Russia
[3]*Saratov State National University, Scientific Bacteriological Laboratory, Saratov, Russia*

Backgrounds

The obligatory intracellular bacterium *C. trachomatis* (CT) is primary known as the causative agent of Chlamydia genital infection in humans. However, some studies have reported that CT was proved to be detected in aborted livestock including cattle (Ozbek et al., 2008). Moreover, human biovars of CT is now considered as risk factors and severity of active trachoma associated with familial cattle ownership.

Objectives

The aim of this study was to determine whether CT could be detected in aborted cattle suspected in chlamydial infection.

Methods

Sera and blood from 21 aborted breeding cows were tested for either specific chlamydial antibody in ELISA or DNA in real-time PCR (InterLabService&Fractal-Bio, Russia) and microscopically. Additionally, each specimen was examined for the presence of CT-specific plasmid followed by PCR target sequencing, as well as PCR testing for *Campylobacter/Listeria/Salmonella/Brucella/Leptospira spp/Toxoplasma gondii/Yersinia pseudotuberculosis*.

Conclusions

We found specific chlamydial antibody in sera of 21/21 cows (100%). No specific DNAs of four the most common Chlamydia species, namely *Chlamydia psittaci/Chlamydia felis/Chlamydia abortus/Chlamydia caviae* were detected. However, detectable concentration of DNA for the presence of CT-specific plasmid target was easily revealed in 8/21 cows (38.1%) that was (8/8, 100%) confirmed by sequence. These PCR&sequencing results were strongly correlated with those obtained by both ELISA and microscopy. No DNA of other pathogens that in known as causative agent for common abortogenic infections were detected. We believe that *C. trachomatis* could play a certain role in zoonotic chlamydial infection as either a single pathogen or co-infection with other Chlamydia which is difficult to detect due to their low concentration in clinical specimens.

FEMS7-1218
Pathogens / Pathogenicity

DETECTION, IDENTIFICATION AND DIVERSITY OF XANTHOMONAS ARBORICOLA PV. JUGLANDIS BY MULTIPLEX PCR AND DOT BLOT HYBRIDIZATION USING NINE DNA MARKERS

C. Fernandes[1,2,3], P. Albuquerque[1], R. Sousa[4], L. Cruz[3,5], F. Tavares[1,2]
[1]CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos- InBIO- Laboratório Associado- Universidade do Porto, MDE - Microbial Diversity and Evolution, Vairão, Portugal
[2]FCUP - Faculdade de Ciências- Universidade do Porto- Rua do Campo Alegre s/nº, Departamento de Biologia, Porto, Portugal
[3]INIAV - Instituto Nacional de Investigação Agrária e Veterinária- Av. da República- Quinta do Marquês, UEIS-
SAFSV - Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal, Oeiras, Portugal
[4]INIAV - Instituto Nacional de Investigação Agrária e Veterinária- Polo de Alcobaça- Estrada de Leiria, ENFVN - Estação Nacional de Fruticultura Vieira Natividade, Alcobaça, Portugal
[5]Bioisi - Biosystems & Integrative Sciences Institute- Campo Grande, Faculdade de Ciências- Universidade de Lisboa, Lisboa, Portugal

Backgrounds

Xanthomonas arboricola pv. *juglandis* (*Xaj*) is a plant pathogen responsible for bacterial blight in walnut trees (*Juglans regia*), and also associated with brown apical necrosis and vertical oozing canker. Detection and identification of *Xaj* are still carried out mainly via culture-based methods, which lack the specificity and time-efficiency characteristic of DNA-based methods. Despite previous efforts, *Xaj*-specific DNA-based detection and identification assays are currently unavailable.

Objectives

This work focused on the development and validation of culture-independent methods for rapid and efficient detection and identification of *Xaj*. For this purpose, *Xaj*-specific DNA markers were selected and applied in PCR- and hybridization-based assays.

Methods

Nine *Xaj*-specific markers were selected using dedicated *in silico* approaches. The use of a high throughput dot blot platform allowed to validate the specificity of the markers, and also to determine strain-specific hybridization patterns. The application of the most promising markers in a multiplex PCR allowed to efficiently detect *Xaj* in naturally infected walnut leaves and fruit samples.

Conclusions

The selected markers were shown to be useful in both hybridization and PCR-based assays. While the obtained hybridization patterns allowed prompt identification of different *Xaj* clonal lineages, the multiplex PCR was validated as an effective culture-independent method for detection of *Xaj* in symptomatic walnut field samples. Ultimately**,** the proposed methods might be particularly useful for routine phytosanitary screenings, to disclose alternative host species and the pathogen lifecycle outside the plant hosts, and, attending to the strain-specific profile of the hybridization patterns, to assess *Xaj* diversity and epidemiology.

FEMS7-0675
Pathogens / Pathogenicity

HOST-AEROMONAS INTERACTION: INFLAMMATORY RESPONSE IN THP-1

A. Fernández Bravo[1], *M.J. Figueras*[1]
[1]*Universitat Rovira i Virgili, Ciencias Médicas Básicas, Reus Tarragona, Spain*

Backgrounds

Case reports have identified *Aeromonas* as an emerging pathogen that cause a wide spectrum of diseases in humans, mainly gastroenteritis, septicemia and wound infections. In spite of the worldwide distribution of *Aeromonas*, its relevance to human health and the way in which the pathogen causes septicemia and death remains poorly understood, besides little is known about its interaction with the human immune system. Monocytes act as first defense against bacterial infections playing a critical role in recognizing and responding to these pathogens. However, so far the impact of *Aeromonas* on human monocytes has not been investigated.

Objectives

To investigate the effect of *Aeromonas* on human monocytes.

Methods

As a first step we have designed an *in vitro* model of infection using a cell line of human monocytes (THP-1) and representative strains of clinical and environmental *Aeromonas*. We tested different infectious doses and incubation times, including in all the experiments a well know *Vibrio vulnificus* strain (CECT 4999) as positive control.

Conclusions

Preliminary results demonstrated that *Aeromonas* showed a tendency to activate a strong immune response in monocytes, transcriptional analysis showed an upregulation in the expression of a variety of immune-related genes including those involved in pathogen recognition, cytokines, chemokines and apoptosis. The latter genes were clearly expressed as derived from the observed degree of cell damage and apoptosis. To determine more accurately these findings, we are studying more strains to clarify if there exists a distinct immune response in different *Aeromonas* species that could explain their differential prevalence in human infections.

FEMS7-1311
Pathogens / Pathogenicity

NEW ANTI-BIOFILM PERSPECTIVES OF THE EUGENOL FOR BIOMEDICAL APPLICATIONS

M.C. Fernández Calderón[1,2], M.T. Blanco Roca[1,2], M.A. Galán Ladero[1], M.T. Blanco Blanco[1], A.C. Gómez García[1,2], C. Pérez Giraldo[1,2]
[1]Faculty of Medicine. University of Extremadura., Biomedical Sciences, Badajoz, Spain
[2]Biomedical Research Centre in Bioengineering Biomaterials and Nanomedicine Network, CIBER-BBN, Badajoz, Spain

Backgrounds

Biomaterials associated infections have a great impact in terms of morbidity, mortality and medical costs. *Staphylococcus epidermidis*, is the microorganism most frequently isolated in these infections, due to exhibits resistance growing in biofilm. Eugenol (E) is a phenolic derivative with analgesic and anti-inflammatory properties; also reduce pain in adjacent tissues. The associating with the mucolytic N-acetylcysteine (NAC) will be enables better therapeutic applications.

Objectives

Evaluate effectiveness of E + NAC for the control of biofilm-associated infections.

Methods

The minimum inhibitory concentration (MIC) of the E alone or in combination with NAC against two biofilm producing strains, *S. epidermidis* ATCC 35984 and ATCC 35983 was determined. The influence of subhinhibitory concentrations (sub-MIC) on the growth and biofilms formation was evaluated. The relationship between these two parameters by "slime index" (SI) was also calculated.

Results: The combination of both compounds did not modify the MIC for any of the strains (MIC = 2 mg / ml) compared to value of then alone. Biofilm production by the highly biofilm-producing strain, ATCC 35984, was increased in the presence of E, even though the growth remained the same or decreased. However, the association of E + NAC reduced the biofilm formation and the IS decreased up to 84.3% with respect to control.

Conclusions

The E + NAC association presented a high capacity to decrease the *S. epidermidis* biofilm formation, which makes them an anti-biofilm alternative for the biomaterials-associated infections control.

Acknowledgments: Ministerio de Economía y Competitividad (MAT2015-63974-C4-4-R), Junta de Extremadura (GR15025) and FEDER funds.

FEMS7-1759
Pathogens / Pathogenicity

PRESENCE OF VIRULENCE FACTORS IN STRAINS OF CANDIDA KRUSEI COLLECTED FROM HEMOCULTURES. STUDY COMPARED TO CANDIDA ALBICANS.

M.A. Galán-Ladero[1], M.T. Blanco-Blanco[1], M.C. Fernández-Calderón[1,2], M.T. Blanco[1,2], C. Pérez-Giraldo[1,2], A.C. Gómez-García[1,2]
[1]University of Extremadura, Biomedical Sciences- Microbiology, Badajoz, Spain
[2]Biomedical Research Center, Bioengineering- Biomaterials and Nanomedicine, Badajoz, Spain

Backgrounds

Candida krusei cause candidemia although with low incidence.

Objectives

In this study we evaluated virulence factors as Cellular Surface Hydrophobicity (CSH), Polystyrene Adherence (PA), Biofilm Formation (BF) and Secreted aspartyl-proteases (Saps) in *C. krusei*, compared to *Candida albicans*

Methods

C. krusei (N=3) and *C. albicans (N=16)* collected from hemocultures during two years were used. Two *C. krusei* strains were isolated from oncohaematologic patients and one from a neutropenic neonate

Virulence factors: HSC was assessed by the MATH method (3). *PA* on microtiter-plates with PBS yeast suspension (1). *BF* on microtiter-plates with RPMI-1640 cultures (4). *Saps activity* on plates with YCB-BSA medium evaluated by Pz index (2).

CSH: All *C. krusei* were hydrophobic with 71.6±14.4% CSH vs 6.8±4% in *C. albicans*

PA: *C. krusei* and *C. albicans* were adherent, both with similar adherent layer (OD: 0.06-0.07)

BF: All *C. krusei* tested were non-biofilm producing, giving identical OD to PA, while *C. albicans* BF achieves a 10-fold OD (0.63± 0.24)

Saps: *C. krusei* strains do not have Saps activity, unlike high activity of *C. albicans*

Conclusions

Virulence factors constitute the mechanisms used to avoid host defences. *C. krusei* are adherent, related to hydrophobicity. The absence of other factors such as non-BF ability, nor protease activity, suggests low virulence levels. This is consistent with the presence of infections in immunocompromised patients

Acknowledgments Junta de Extremadura (GR15025) and FEDER funds

References

(1)-Blanco et al *Microbios*1997,89:23;(2)-Galán-Ladero et al *Med Mycol* 2010,48:207;
(3)-Hazen *Infect Immun*1988,56:2521;(4)-Ramage et al *Rev Iberoam Micol* 2001,18:163;

FEMS7-1296
Pathogens / Pathogenicity

THE ANTIMICROBIAL PEPTIDE POLYMYXIN B NONAPEPTIDE ENHANCES BOTH EFFLUX PUMP INHIBITORS AND ANTIBIOTICS AGAINST PLANKTONIC AND BIOFILM-FORMING CELLS OF MEXAB-OPRM OVEREXPRESSING PSEUDOMONAS AERUGINOSA STRAINS

R. Ferrer-Espada[1], S. Sánchez-Gómez[2], B. Pitts[3], P.S. Stewart[3], G. Martínez de Tejada[1]
[1]University of Navarra, Microbiology and Parasitology, Pamplona- Navarra, Spain
[2]Bionanoplus S. L., -, Noain- Navarra, Spain
[3]Montana State University, Center for Biofilm Engineering, Bozeman- Montana, USA

Backgrounds

Resistance to antibiotics poses a “major global threat” to public health according to the World Health Organization. The increasing emergence of bacterial clones insensitive to these drugs greatly limits the therapeutic options and highlights the urgent need to develop novel and effective treatments for infections caused by these microorganisms. We hypothesized that subinhibitory concentrations of a permeability-increasing antimicrobial peptide (AMP) could enhance the activity of efflux pump inhibitors (EPIs) and sensitize bacteria to antibiotics that are efflux substrates.

Objectives

Our objective was to measure the ability of a model AMP, polymyxin B nonapeptide (PMBN), to synergize with antibiotics such as aztreonam, ceftazidime, doxycycline, levofloxacin, piperacillin or azithromycin in the presence of EPIs (1-(1-naphthylmethyl)-piperazine (NMP) or Phenylalanine-Arginine β-Naphthylamide (PAβN)). Tests were performed using planktonic and biofilm-forming cells of MexAB-OprM overexpressing *Pseudomonas aeruginosa* strains.

Methods

To characterize the antimicrobial activity of the combinations, we used conventional MIC testing, checkerboard analysis, growth inhibition curves and anti-biofilm activity measured by viable counts on biofilms grown in a reactor operating in the turbulent flow regime.

Conclusions

We demonstrated for the first time that PMBN greatly enhanced EPIs activity against two different MexAB-OprM overproducing strains and sensitized them to all tested antibiotics. The selected triple combination (Azithromycin/PAβN/PMBN) caused a 10 million fold reduction in biofilm forming cells. This phenomenon can be exploited to sensitize efflux pump overexpressing strains of *P. aeruginosa* to antibiotic substrates of those mechanisms of resistance.

FEMS7-1470
Pathogens / Pathogenicity

IDENTIFICATION OF GENOMIC ISLANDS IN CHILEAN PISCIRICKETTSIA SALMONIS STRAINS AND ANALYSIS OF GENE EXPRESSION INVOLVED IN VIRULENCE

J. Figueroa[1], *F. Lagos*[1], *C. Cartes*[1], *D. Haussmann*[1]
[1]*Instituto de Bioquímica & Microbiología., Biochemistry & Microbiology, Valdivia, Chile*

Backgrounds

Piscirickettsiosis is a septicemic disease that affects salmonids. *P. salmonis* is a non-encapsulated, Gram-negative immobile pleomorphic, but generally coccoid bacteria with a diameter between 0.2 and 1.5 microns and is a facultative bacteria.

Objectives

The aim of this study was to bioinformatically identify genomic islands in genomes from different chilean strains of *P. salmonis*, in order to later select probable genes involved in the infective process *in vitro*.

Methods

We identified, characterised and bioinformatically analysed genomic islands in field strains of *P. Salmonis*, using the bioinformatic software PIPS, that uses the characteristics of the islands of pathogenicity to identify them.

Conclusions

We analysed 9 partially sequenced genomes in different new field strains, and compared them with the LF-89 genome, selecting a genomic island present in all of them. We then evaluated the relative expression of three genes present in that island. From the obtained results, we conclude that the expression of the *tcf* gene is directly proportional to the cytopathogenicity *in vitro* of the bacteria; the product of the *dnsa* gene could contribute to its pathogenicity. The product of the gene *liso* is necessary for the virulence process and could have functions in early stages of infection. Regarding the strains, the IBM-040 strain showed a significant increase in the expression of all the genes in the study. Contrarily, LF-89 only presented a significant increase in expression of the gene *liso*, which correlates with the cytopathogenicity in vitro observed in the SHK-1 cells.

Fondecyt-1130069 and FONDAP-INCAR-15110027

FEMS7-0294
Pathogens / Pathogenicity

VIRULENCE-ASSOCIATED TRAITS OF ACHROMOBACTER SPECIES OF CF AND NON-CF ORIGIN

B. Filipić[1,2], M. Malešević[2], Z. Vasiljević[3], J. Lukić[2], K. Novović[2], M. Kojić[2], B. Jovčić[2,4]
[1]Faculty of Pharmacy- University of Belgrade, Department of Microbiology and Immunology, Belgrade, Serbia
[2]Institute of molecular genetics and genetic engineering- University of Belgrade, Laboratory for molecular microbiology, Belgrade, Serbia
[3]Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic“, Laboratory for clinical microbiology, Belgrade, Serbia
[4]Faculty of Biology- University of Belgrade, Biochemistry and Molecular Biology, Belgrade, Serbia

Backgrounds

Achromobacter spp. are oportunistic pathogens that can lead to various infections in immunocompromised individuals as well as in cystic fibrosis (CF) patients. Beside the fact that incidence of achromobacterial disease continues to rise, little is known about virulence factors of this species. From 2012 to 2015, we collected 69 clinical isolates of *Achromobacter* spp. from patients with CF (CF isolates, n=32) and patients receiving care for other health conditions (non-CF isolates, n=37) in order to examine their virulence traits.

Objectives

The main goal of this study was to investigate virulence potential of both CF and non-CF *Achromobacter* spp. isolates through caracteristics such as ability of biofilm formation, motility and binding affinity to mucin, collagen and fibronectin.

Methods

Motility of isolates was examined on plates with low content of agar, while biofilm formation assay was performed in 96-well microtiter plates. Mucin, collagen and fibronectin binding abilities were also tested in microtiter plates with wells coated with a suitable substrate.

Conclusions

CF isolates from this study were less motile compared to non-CF isolates. Diminished motility among our CF isolates probably serves as an competitive advantage in establishing an infection in the airways. About one third of the isolates were classified as strong biofilm producers, and the proportion of CF and non-CF isolates with the ability to form biofilm was almost identical. At the other hand, CF group of isolates exhibited higher binding affinity to mucin, collagen and fibronectin, making CF isolates apparently more virulent compared to the non-CF group.

FEMS7-2736
Pathogens / Pathogenicity

FEATURES OF IMMUNOGENIC PROPERTIES OF VACCINE FRANCISELLA TULARENSIS 15 NIIEG DERIVATIVE STRAINS

V. Firstova[1], V. Pavlov[2], A. Pinchuk[1], O. Kalmantaeva[1], T. Kombarova[3], G. Vakhromeyeva[4], A. Mokrievich[5], R. Mironova[6], I. Dyatlov[7]
[1]State Research Center for Applied Microbiology & Biotechnology, Infection Immunity, Obolensk, Russia
[2]State Research Center for Applied Microbiology & Biotechnology, Microbiology of Tularemia, Obolensk, Russia
[3]State Research Center for Applied Microbiology & Biotechnology, Biological investigation, Obolensk, Russia
[4]State Research Center for Applied Microbiology & Biotechnology, Tularemia microbiology, Obolensk, Russia
[5]State Research Center for Applied Microbiology & Biotechnology, Particularly dangerous infections, Obolensk, Russia
[6]State Research Center for Applied Microbiology & Biotechnology, Tularemia infection, Obolensk, Russia
[7]State Research Center for Applied Microbiology & Biotechnology, Director, Obolensk, Russia

Backgrounds

Live tularemia vaccine requires further improvement in connection with instability and residual reactogenicity. *iglC* and *sodB* genes are encoded products which necessary for survival and multiplication of bacteria inside the phagocytes and inactivation of oxygen and nitrogen free radicals. Derivative strains of *F.tularensis* 15NIIEG with one copy of the *iglC* gene deletion, *recA* gene deletion (to reduce the amounts of recombination) and/or with a modified *sodB* gene were constructed.

Objectives

Comparison between mice groups immunized with various vaccine strain derivatives in the protection level against challenge with *F.tularensis* Schu and lymphocytes specific activation in response to restimulation with *F.tularensis* antigens.

Methods

BALb/c mice were immunized with vaccine strain or its derivatives and then on 28 day were challenged with *F.tularensis* Schu. Blood and spleens from five mice of each immunised group were obtained. Lymphocytes were incubated in the presence of *F. tularensis* antigens with subsequent cytometric and ELISA analysis of activation and co-stimulatory molecules and cytokine activities of cells.

Conclusions

Comparative studies showed that maximum protection against challenge with *F.tularensis* Schu was observed in mice previously immunized with *F.tularensis* 15/10Δ23sodBrecA. In this mice group was found the most significant IFN-γ secretion by T-helpers and B-lymphocytes in response to restimulation by antigens of *F. tularensis*. The level of lymphocyte proliferative activity and the number of antibodies to *F. tularensis* antigens in all animal groups were identical. These data confirm the importance of IFN-γ in protection against infection with *F.tularensis* Schu - highly virulent for humans and other mammals.

FEMS7-1326
Pathogens / Pathogenicity

EFFECT OF PROLONGED ANTIBIOTIC USE ON THE ADULT GUT RESISTOME

E. Fletcher[1], L. Imamovic[1], M. Ellabaan[1], E. van der Helm[1], W. van Schaik[2], M. Sommer[1]
[1]Technical University of Denmark, Novo Nordisk Foundation Centre for Biosustainability, Lyngby, Denmark
[2]University Medical Center Utrecht, Department of Medical Microbiology, Utrecht, The Netherlands

Backgrounds

Antibiotics play a major role in treating minor infections and are also used to decontaminate the digestive tract of patients on admission at the hospital to prevent the risk of infection during surgery.

Objectives

Antibiotic use, however, has profound effects on the gut microflora resulting in the selection of bacteria that are resistant to the antibiotics administered. We, therefore, investigated changes in the resistome of adults given prolonged exposure to multiple antibiotics.

Methods

We used functional metagenomic selections on 14 antibiotics to identify known and novel antibiotic resistance genes that constitute the gut resistome of adults admitted at the Intensive Care Unit (ICU). Functional annotation of the patients' resistomes revealed the presence of 47, 89 and 99 unique gene types found in the CARD, ARDB and Resfams databases respectively. Beta-lactamases and aminoglycosides represented the most abundant resistance gene class. This can be linked to prolonged exposure of adult ICU patients to beta-lactam and cephalosporin drugs as well as selective digestive tract decontamination (SDD) treatment containing aminoglycosides. By comparing this resistome to an *in silico* analysis of two other human gut resistomes we confirmed that antibiotic use significantly reduced the genetic diversity of the resistome as specific resistance genes were selected by the antibiotics administered. By further exploring the resistome of the adult ICU patients, we also identified previously uncharacterized (novel) genes that conferred resistance to beta-lactams, aminoglycosides and fosfomycin.

Conclusions

Our study, therefore, presents a comprehensive description of the effect of prolonged antibiotic use on the adult gut resistome indicating that antibiotic use alters the diversity of the gut resistome.

FEMS7-2067
Pathogens / Pathogenicity

VIBRIO HARVEYI: AN EMERGENT PATHOGEN IN MARINECULTURE

O. Tomás[1], A. Llorens[2], D. Liz[2], C. Amaro[2], B. Fouz[2]
[1]University of Valencia, Department of Microbiology and Ecology and ERI Biotecmed, Burjasot-Valencia-46100., Spain
[2]University of Valencia, Department of Microbiology and Ecology and ERI Biotecmed, Burjasot- Valencia- 46100., Spain

Backgrounds

In the last two years, *Vibrio* strains have been isolated from epizootics occurred in European sea bass (*Dicentrarchus labrax*) farms located on the Iberian Peninsula coast. Given that fish had been vaccinated against *V. anguillarum*, the emergence of another pathogenic vibrio was occurring. Within the *Vibrio* genus, *V. harveyi* is a recognized secondary pathogen of marine vertebrates and invertebrates, including important cultured species.

Objectives

i) To identify and characterize the fish pathogenic isolates; ii) To study their virulent potential both “in vitro” and ” in vivo” conditions.

Methods

We have identified the *Vibrio* isolates by using phenotypic and genotypic (including specific PCR protocols) tests and have characterized their production of exoenzymes involved in virulence. Moreover, we have assessed their ability to survive in fish and human plasma and their virulent potential for different hosts.

Conclusions

Fish pathogenic isolates, identified as *V. harveyi*, successfully multiplied in plasma of sea bass and were virulent for this species. Furthermore, all strains gave positive results for two fish virulence plasmid markers (*vep20* and *vep07* genes*)* well characterized in the close fish pathogen *V. vulnificus*. In contrast, all strains did not survive in human plasma and were not virulent for mouse. Therefore, *V. harveyi* could be considered an emergent primary pathogen for marine fish that would not represent a risk for Public Health. In order to minimize economic losses in Aquaculture industry, the development of novel specific vaccines would be convenient.

FEMS7-1027
Pathogens / Pathogenicity

MOLECULAR EPIDEMIOLOGY OF EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING ESCHERICHIA COLI CAUSING BACTERAEMIA IN SPAIN

S.C. Flament-Simon[1], R. Mamani[1], V. García Menéndez[1], C. López[1], M.P. Alonso García[2], J.E. Blanco Álvarez[1], J. Blanco Álvarez[1]
[1]Universidad de Santiago de Compostela, Microbioloxia e Parasitoloxia, Lugo, Spain
[2]Hospital Universitario Lucus Augusti, Microbiology, Lugo, Spain

Backgrounds

Pathogenic *E. coli* are the predominant cause of Gram-negative bloodstream infections and bacteraemia caused by extended-spectrum β-lactamase-producing *E. coli* (ESBLEC) is associated with adverse patient outcomes and increased costs.

Objectives

The objective of this study was to assess the prevalence and molecular epidemiology of ESBLEC causing bacteraemia in a Spanish Hospital over a 23-Year Period (1989 to 2011).

Methods

During 1989 to 2011, 3,260 bacteraemic *E. coli* isolates were studied and 96 (2.9%) ESBLEC were detected and characterized. ESBL enzymes, phylogenetic groups, clonotypes (CHs), sequence types (STs), serotypes and virulence genes were determined.

The most prevalent ESBLs were CTX-M-15 (41.7%), CTX-M-14 (37.5%) and CTX-M-1 (8.3%). Forty-four (45.8%) isolates belonged to phylogroup B2, 18.8% to group B1, 11.5% to group A, 11.5% to group E, 6.3% to group F, 5.2% to group C and 1.0% to group D. Thirty-six STs and 40 CHs were identified. However, 41 (42.7%) of the 96 ESBLEC isolates belonged to the clonal group ST131. The ST131 isolates exhibited a significantly higher virulence score compared with the non-ST131 isolates. All 41 ST131 isolates belonged to phylogroup B2 and 39 belonged to serotype O25b:H4. The other two ST131 isolates belonged to serotypes O16:H5 and ONT:H5. The *H*30Rx subclone accounted for most ST131 isolates (35 CTX-M-15 isolates). Three ST131 isolates belonged to subclone H22 and two isolates to subclone H41.

Conclusions

ST131 was the most antimicrobial-resistant sequence type and the *H*30Rx subclone was responsible for the significant increase of ESBLEC isolates since 2006.

FEMS7-0695
Pathogens / Pathogenicity

GENOME SEQUENCE OF ST131 ESCHERICHIA COLI STRAIN ENCODING COLISTIN RESISTANCE DETERMINANT MCR-1 ISOLATED FROM PORK MEAT: COMPARISON WITH HUMAN CLINICAL ST131 ISOLATES

I. García Meniño[1], M. Sergeant[2], D. Díaz Jiménez[1], A. Herrera Estévez[1], S. Viso González[1], L. Costoya Seco[1], M. Blanco Álvarez[1], A. Mora Gutiérrez[1]
[1]Universidad de Santiago de Compostela, Microbioloxía e Parasitoloxía, Lugo, Spain
[2]University of Warwick- Warwick Medical School, Microbiology and Infection Unit, Coventry, United Kingdom

Backgrounds

Sequence type 131 (ST131) is one of the successfully spread *Escherichia coli* groups among extraintestinal pathogenic *E. coli* (ExPEC). The plasmid-mediated colistin resistance mechanism MCR-1 has been recently described in *E. coli* isolates. Presently, only four ST131 *E. coli* strains carrying *mcr-1* have been reported.

Objectives

The aim of this study was to analyze and compare a colistin-resistant *Escherichia coli* ST131 isolated in Spain in 2011 from pork meat with human clinical isolates.

Methods

By conventional molecular typing, the *mcr-1* carrying *E. coli* isolate LREC-109 was O25b:H4-B2-ST131/PST43, *fimH298 papGIII cdtB iutA iucD iroN kpsMII-K5 cvaC iss traT ibeA malX usp*.

LREC-109 and 11 highly similar ST131 isolates (PFGE similarity >84,6%) were whole genome sequenced. In the Minimum Spanning Tree of the core genome analysis based on the cgMLST scheme from Enterobase (https://enterobase.warwick.ac.uk/), 7 human clinical isolates included in the analysis showed 37-131 differences compared to LREC-109, and 124-160 SNPs in the core genomic regions present in 90% of the 12 compared genomes. PlasmidFinder and ResFinder tools (http://www.genomicepidemiology.org/) showed the presence in LREC-109 of IncHI2 (ST4) and IncF (F2:A-:B-) plasmids, as well as several resistance genes.

Interestingly, we have detected in Enterobase a human clinical ST131 genome from an *E. coli* isolated in UK in 2010, showing only 10 differences in the cgMLST comparison with LREC-109, and 51 SNPs.

Conclusions

We report the presence in Spain of a colistin-resistant ST131 strain from pork meat, carrier of a wide number of virulence genes, MDR, and sharing close relationship with human isolates.

FEMS7-0400
Pathogens / Pathogenicity

ENTEROCOCCUS FAECIUM VESICULOGENESIS: IS A MECHANISM TO EXPORT PERIPLASMIC PROTEINS

M. Garcia Solache[1], *L.B. Rice*[1]
[1]*Rhode Island Hospital, Medicine, Providence, USA*

Backgrounds

Background: *Enterococcus faecium* is an important agent of nosocomial infections. Many clinical *E. faecium* strains are resistant to β–lactam antibiotics and vancomycin. Resistance to β–lactams is mainly attributed to Pbp5, a low-affinity Class B penicillin-binding protein, but other proteins, such as the recently identified P_5AP, also participate. Although the P_5AP sequence suggests a cytoplasmic location, we had localized it extracellularly associated with Pbp5. The presence of P_5AP in the extracellular milieu suggests a non-classical secretion mechanism.

Objectives

Objectives Identify the mechanism of P_5AP export.

Methods

Methods Membrane-derived vesicles (MV) were purified from E faecium strain C68. The protein content of MV was analysed with Mass Spectrometry. MV preparations underwent P_5AP and Pbp5 western blot. Fluorescently labelled P_5AP was also tracked *in vivo*.

Conclusions

Conclusions: We identified P_5AP as part of MV cargo along with Pbp5. Mass spectrometry indicated that P_5AP was one of the most abundant proteins in MV along with the penicillin binding proteins Pbp5, PonA, Pbp2B and the cell division protein FtsZ. The fluorescently labelled P_5AP localised mainly to the poles and septum. The identification of a novel mechanism for export of P_5AP and other proteins to the periplasmic space might suggest new targets for drug development to help curb antimicrobial resistance.

FEMS7-3018
Pathogens / Pathogenicity

HIGHLY POTENT SYNERGISTIC COMPOSITION OF AN OIL AND HERBAL EXTRACT FOR THERAPEUTIC APPLICATIONS.

V. Gautam[1], *S. Kaur*[1], *L. Singhal*[2], *P. Ray*[1]
[1]*Post Graduate Institute of Medical Education & Research, Medical Microbiology, Chandigarh, India*
[2]*Government Medical College & Hospital, Mocrobiology, Chandigarh, India*

Backgrounds

The present invention discloses a synergistic composition of herbs based oil (Oil based on Herbs A, OHA) and Herb B (HB) whole plant extract with drastically enhanced efficacy as compared to the ingredients alone. In India, these oil & herb are used for non-healing wounds, burns, sinus, blisters, abscess, bite wounds etc. and also applied over infected skin diseases such as hand-foot-and-mouth disease, eczema, syphilis etc.

Objectives

In this study, we checked the anti-bacterial activity of both the herb and oil individually and in combination against four most common non-fermenters.

Methods

The *in vitro* anti-bacterial activity of methanolic extract of OHA and HB was checked against *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), *Stenotrophomonas maltophilia* (ATCC 13637) and *Burkholderia cepacia* complex (ATCC 25609). Minimum inhibitory concentrations (MICs) & checkerboard assay of MHA and HB were determined using micro broth dilution method in ATCC as well as clinical strains of all the four non-fermenters.

Conclusions

MIC of OHA against the four non-fermenters was found to be 5,000 µg/ml while MIC of HB was 4,500 µg/ml – 9,000 µg/ml respectively in ATCC as well as clinical strains of all these four NFGNB. Surprisingly, no growth at all was observed in checkerboard with different concentrations combinations of MHA and HB. A highly potent antimicrobial effect was obtained after using combinations of MHA and HB resulted in reduction of MIC, approx. 250-500 fold for OHA (i.e. 4,500-9,000/17.5); and approx. 7 million fold for HB (i.e. 5,000/0.0007). It has been patented (Patent no. 201611030748, Intellectual property India).

FEMS7-2227
Pathogens / Pathogenicity

CHARACTERISTICS OF POLISH PENICILLIN-RESISTANT CLINICAL ISOLATES OF ENTEROCOCCUS FAECALIS

I. Gawryszewska[1], D. Żabicka[2], K. Malinowska[2], W. Hryniewicz[2], E. Sadowy[1]
[1]National Medicines Institute, Department of Molecular Microbiology, Warsaw, Poland
[2]National Medicines Institute, Department of Epidemiology and Clinical Microbiology, Warsaw, Poland

Backgrounds

Enterococcus faecalis is natural part of human microflora but also a common cause of hospital infections. Nosocomial *E. faecalis* strains are predominantly grouped in high-risk enterococcal clonal complexes (HiRECCs). Penicillin-resistant isolates of *E. faecalis* remain rare, however the number of such isolates reported to the National Reference Centre for Susceptibility Testing recently increases.

Objectives

The objective was to perform analysis of Polish penicillin-resistant hospital isolates of *E. faecalis* to elucidate the path of emergence of this strains.

Methods

Antimicrobial susceptibility was evaluated by broth microdilution and Etest. The presence of pheromone-responsive plasmids was investigated by the clumping test, and PCR detection of plasmid-specific genes. Biofilm formation was determined as adherence to polystyrene plates. The relatedness among isolates and identification of clonal complexes was established by multilocus sequence typing (MLST) and eBURST analysis with the data from the MLST database. The β-lactamase production was examined by a cefinase test. The penicillin-binding protein *pbp5* gene was sequenced to search for putative mutation(s).

Conclusions

Polish penicillin-resistant clinical *E. faecalis* isolates were multidrug resistant. The majority of isolates formed biofilm, important for virulence and persistence in hospital environment. While the production of aggregation substance was observed for few isolates, genes of pheromone plasmids were commonly detected, indicating the potential of such strains to disseminate resistance determinants both associated with such plasmids and by mobilization of chromosomal genes. Penicillin resistance emerged in HiRECCs due to *pbp5* mutations. The acquisition of penicillin resistance by HiRECCs may be the next step in the evolution of *E. faecalis* as nosocomial pathogen.

FEMS7-1527
Pathogens / Pathogenicity

ERWINIA AMYLOVORA: A GLOBAL PHAGE HOST RANGE STUDY USING STANDARDIZED QUANTITATIVE PCR

S. Gayder[1], A. Castle[2], A. Svircev[3]
[1]Brock University, Centre for Biotechnology, St. Catharines, Canada
[2]Brock University, Department of Biological Sciences, St. Catharines, Canada
[3]Agriculture and Agri-Food Canada, Science and Technology Branch, Vineland, Canada

Backgrounds

Erwinia amylovora is a pathogen of commercially grown pear and apple trees. Bacteriophages, isolated from orchard soils, were previously combined with the epiphyte *Pantoea agglomerans* to form an effective biological control agent.

Objectives

A standardized quantitative PCR (qPCR) host range protocol was developed to study the ability of 5 *Myoviridae* and 5 *Podoviridae* phages, isolated in Canada, to replicate in 99 *E. amylovora* and 30 *P. agglomerans* isolates from North America, Europe, Northern Africa, and New Zealand.

Methods

A standard plasmid, containing amplicons from the bacteriophages, was designed to relate the cycle threshold in qPCR to DNA copy number for rapid phage quantification. All bacterial isolates were inoculated with phage at a low multiplicity of infection and incubated for 8 h. This standardized qPCR was used to determine each host's ability to produce phage progeny.

Conclusions

The results showed that while all *E. amylovora* hosts could replicate phage to some extent, 10 isolates from the west coast of North America and one from Poland could only replicate one family of phage. The best hosts increased phage titre up to 7 log units, while these poor hosts increased the titre by less than one log. Additionally, only three *P. agglomerans* hosts could replicate phages from both families, with most isolates being infected only by *Myoviridae* phages. These results showing a broad host range of these 10 tested phages on the pathogen *E. amylovora* and narrow host range on *P. agglomerans* could be used to select the ideal components in the carrier-phage biological control agent.

FEMS7-0415
Pathogens / Pathogenicity

ADHERENCE OF CANDIDA ALBICANS TO METHYL (POLYMETHYL METHACRYLATE) SURFACES WHEN SUBMITTED TO ATMOSPHERIC-PRESSURE COLD PLASMA

E. Stieven[1], C. Bosi[1], G.C. Lain[2], A. Conde[1], R. Elsemann[1], E. Elsemann[1], J.F. Cano[3], A. Gazzoni[1]
[1]Centro Universitário da Serra Gaúcha, School of Dentistry, Caxias do Sul, Brazil
[2]Plasmar Tecnologia, Departamento de Desenvolvimento, Caxias do Sul, Brazil
[3]Universitat Rovira i Virgili, Mycology Unit- Medical School and IISPV-, Reus, Spain

Backgrounds

Development of methods that modify the denture base surface properties to prevent the adherence of *C. albicans* be a significant advancement in the prevention of Candida-associated denture stomatitis.

Objectives

To investigated the effects of atmospheric pressure cold plasma treatment in early *C. albicans* adherence on heat-polymerized acrylic resin.

Methods

Thirty polymethyl methacrylate specimens were made from heat-polymerized acrylic resin base material. Specimens were prepared as square shaped with a width of 10 mm and 4 mm thickness. All pieces were polished in the upper portion, in low rotation with felt disc. For plasma application, Teflon tube with a mixture gas of argon (98%) and oxigen (2%) was used at a flow rate of 5L/min. Specimens were grouped as follows: fifteen specimens were non-treated (Group A), while the remaining especimens were treated with cold plasma for 120 seconds (Groups B). *C. albicans* (ATCC 90028) was used for fungal adherence studies. After the plasma treatment, specimens were inoculated with a suspension standardized to a concentration of $1x10^6$ CFU/mL. After 24 hours of incubation, the amount of the yeasts adhering to specimen surfaces was evaluated by CFU.

Conclusions

In Group A was observed mean of fungal adhesion equal to 4.84 (2.0-8.1 ± 1.81). In Group B, an average of adhered microorganisms was 0.28 (0.12-0.6 ± 0.10). When Group A was compared to Group B, there was a significant difference ($P < 0,0001$). Teh use of cold plasma seems to be a promising and convenient strategy to prevent the adhesion of *C. albicans* to acrylic resins.

FEMS7-0697
Pathogens / Pathogenicity

IN VITRO STUDY ABOUT THE ADHERENCE OF CANDIDA ALBICANS TO THREE TYPES OF POLISHING ON COMPOSITE RESIN SURFACES

A. Mondadori Garcia[1], A. Conde[1], C. Boniatti Mussato[1], R. Elsemann[1], E. Elsemann[1], J.F. Cano[2], A. Gazzoni[1]
[1]Centro Universitário da Serra Gaúcha, School of Dentistry, Caxias do Sul, Brazil
[2]Mycology Unit, Medical School and IISPV, Reus, Spain

Backgrounds

Candida albicans is responsible of the exponential growth of *Streptococcus oralis* and *Actinomyces oris* in dental materials as well as of the development of dental caries.

Objectives

To demonstrate the adhesion potential of *C. albicans* on three types of polishing on photopolymerizable composite resin surfaces.

Methods

In light-curing composite resin (Z350XT, 3M®), rectangular-shape samples with dimensions of 10 mm x 5 mm x 30 mm were prepared. They were grouped according to the following criteria: Group A - Sof-Lex sandpaper discs (3M®), Group B - Sof-Lex sandpaper discs (3M®) plus Velvety disc Polifix (TDV®) plus Polishing paste Diamond Excel (FGM®) and Group C - finishing and polishing rubbers for composite resin Jiffy (ULTRADENT®). Specimens were inoculated with a standardized solution of 10^6 CFU/mL of *C. albicans* strain (ATCC 90028). After 24 hours of incubation, the CFU/mL were established for each group. Differences in adherence rates among the groups were compared using one-way ANOVA analysis.

Conclusions

Group A presented a mean of fungal adhesion of $0.03x10^{10}$ (0.013–0.096±0,24). In group B, the adherence was $0,012\ x10^{10}$ (0.008–0.80± 0.02). In group C, the mean of adhesion was $6.50\ x10^{10}$ (5.10–9.50±1.49). Significant difference was observed among groups B and C (P=0.0002). There was significant difference between groups A, B and C with a higher adhesion rates observed on group C (P<0.0001). Increase of surface roughness significantly increases the adhesion rates of *C. albicans* to the surfaces of composite resin, since this microorganism has influence on the exponential growth of *S. oralis- oris* in dental materials.

FEMS7-2651
Pathogens / Pathogenicity

PHYLOGEOGRAPHY AND POPULATION STRUCTURE OF XANTHOMONAS FRAGARIAE TO IDENTIFY SOURCES AND PATHWAYS OF THIS BACTERIAL PHYTOPATHOGEN THROUGH PLANT MATERIAL TRADE

M. Gétaz[1], M. Krijger[2], J. van der Wolf[2], B. Duffy[1], J. Pothier[1]
[1]Zurich University of Applied Sciences ZHAW, Institute of Natural Resource Sciences, Wädenswil, Switzerland
[2]Wageningen UR, Biointeraction and Plant Health, Wageningen, The Netherlands

Backgrounds

Angular leaf spots are caused by *Xanthomonas fragariae* with particular severe effects under protected cultivation with high-density plots aided by high humidity and sprinkler irrigation systems. *X. fragariae* was first observed in the USA in 1960, in Europe in 1970 and then spread worldwide in strawberry growing regions, causing yield loss of harvested fruits.

Objectives

Due to its quick spread, thought to be due to importation of plant material through trade and more generally of human activities, there is a real need for reliable methods accurately discriminating between strains for crop surveillance, outbreak investigation and establishing disease control strategies.

Methods

As relatively low diversity was observed among 59 *X. fragariae* strains of various geographic and time origins, the discrimination power of several molecular markers was determined. Among these markers for source tracking purpose, variable number of tandem repeats (55 VNTRs) were used as an efficient genotyping method, where numbers of repeats acted as molecular clocks with sufficient resolution to discriminate strains. In addition, clustered regularly interspaced short palindromic repeats (2 CRISPRs) were useful markers, also used in epidemiology and host-bacteria surveys, bringing complementary information for genetic diversity characterization and direction of evolution.

Conclusions

This study highlighted the flux of evolution of *X. fragariae* strains and two main origins of evolution that could be responsible for the world-wide invasion of *X. fragariae*. An understanding on population structures of the quarantine pathogen *X. fragariae* and how the disease can emerge and spread over a given geographical region are trivial for bacterial monitoring.

FEMS7-2044
Pathogens / Pathogenicity

ANALYSIS OF THE POTENTIAL INVOLVEMENT OF THE PIL FIMBRIAE IN THE ADHESION OF ENTEROPATHOGENIC ESCHERICHIA COLI (EPEC)

F.S. Castro[1], M.A.M. Vieira[1], B.G. Garcia[1], F.T. Romao[1], D. Yamamoto[1], T.A.T. Gomes[1]
[1]Federal University of Sao Paulo, Microbiology- Immunology and Parasitology, Sao Paulo, Brazil

Backgrounds

Enteropathogenic (EPEC) and enteroaggregative (EAEC) *Escherichia coli* are significant diarrheal agents, which produce localized adherence (LA) (mediated by the Bundle-forming Pilus) and aggregative adherence (AA) patterns on HeLa cells, respectively. We have previously identified EPEC strains producing LA and AA-like patterns concurrently (LA/AA-like+). Transfer of an 80-kb plasmid (pGM80), which bears the *pil* operon that encodes a variant-Pil fimbria from one LA/AA-like+ strain (EC404/03, serotype O119:H6) to a non-adherent *E. coli* (MA3456) yield AA-like+ transconjugants carrying pGM80. However, we have also observed that some LA positive (LA+) O119:H6 strains also carry *pil*.

Objectives

To analyze the involvement of Pil in O119:H6 EPEC adhesiveness.

Methods

Three LA+ strains were compared with EC404/03 (LA/AA-like+) concerning their ability to transfer *pil* by conjugation. Transconjugants were tested for HeLa cells adhesion, plasmid profiles and presence of all 14 *pil* genes. Additionally, expression of five essential Pil-assembly genes (*piLN, pilQ, pilR, pilS* and *pilU*) was compared by qRT-PCR between each LA+ strain and the LA/AA+ strain. All transconjugants were non-adherent and devoid of *pil*. Although the LA+ strains carried all 14 *pil* genes, variable expressions of the five *pil* genes tested were observed between each LA+ strain and the LA/AA+ strain, some of which were repressed while others were overexpressed.

Conclusions

A fine regulation of the *pil* genes is probably required for expression of functional Pil fimbriae. Undergoing molecular studies, and immunolabelling and competition assays with specific antisera will further help to elucidate the involvement of Pil in the EPEC AA-like pattern.

FEMS7-1104
Pathogens / Pathogenicity

THE LEGIONELLA GENUS GENOME: A GLOBAL VIEW OF THE GENUS EVOLUTION

L. Gomez-valero[1,2], C. Rusniok[1,2], G. Schroeder[3,4], D. Carson[3,4], S. Mondino[1,2], A.E. Perez-cobas[1,2], M. Rolando[1,2], S. Reuter[5], J. Dermitas[1,2], J. Crumbach[1,2], S. Descorps-declere[6], G. Frankel[7], S. Jarraud[8,9], E. Hartland[10], C. Buchrieser[1,2]
[1]Institut Pasteur, Genomes and Genetics, Paris, France
[2]CNRS, UMR 3525, Paris, France
[3]MRC Centre for Molecular Bacteriology and Infection- Imperial College, Department of Life Sciences- Imperial College, London, United Kingdom
[4]Centre for Experimental Medicine, Queen's University, Belfast, United Kingdom
[5]University Medical Center Freiburg, Infection Prevention and Hospital Hygiene, Freiburg- Baden-Württemberg, Germany
[6]Institut Pasteur, Hub Bioinformatique et Biostatistique, Paris, France
[7]MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences- Imperial College, London, United Kingdom
[8]International Center for Infectiology Research-École Normale Supérieure de Lyon, Inserm- U1111- CNRS- UMR5308-, Lyon, France
[9]National Reference Centre of Legionella, Hospices Civils de Lyon, Lyon, France
[10]University of Melbourne, Microbiology and Inmunology, Melbourne, Australia

Backgrounds

Legionellosis is a severe pneumonia caused by bacteria belonging to the genus *Legionella*. Currently there are 66 species/subspecies described within this genus but over 95% of Legionnaires' disease cases are caused by only two species: *L. pneumophila* and *L. longbeachae*.

Objectives

The purpose of this work was to carry out the largest genomic comparison done until now at the genus level between different *Legionella* species/strains to decipher the genomic components that are responsible of their different capacities for human infection.

Methods

With this aim we have analysed and compared 80 *Legionella* strains belonging to 58 *Legionella* species-subespecies. We have also developed bioinformatics approaches that allowed us to detect new eukaryotic motifs not described before and to predict new putative effectors in all studied species. Moreover we have validated for selected effectors that they are translocated via the Dot/Icm secretion system. Finally the potential virulence capacity of most of the strains has been explored using the human monocytic cell line THP-1

Conclusions

We found that species with higher clinical incidence also display enhanced infectivity *in-vitro*. Comparative genomics showed that presence of the Dot/Icm secretion system and of many eukaryotic like proteins are universal features of this genus. However, among the over 300 Dot/Icm effectors described for *L. pneumophila*, only 7 belong to the genus core genome. In addition our evolutionary analysis indicated that most of the predicted Dot/Icm effectors in the genus *Legionella* have been incorporated trough gene gain events and that loss events are less frequent.

FEMS7-2095
Pathogens / Pathogenicity

IDENTIFICATION AND CHARACTERIZATION OF DOT/ICM SUBSTRATES WITH EUKARYOTIC CONSERVED DOMAINS IN THE FISH PATHOGEN PISCIRICKETTSIA SALMONIS

F. Gomez[1], O. Arredondo-Zelada[1], F.A. Hanriquez[1], P. Flores-Herrera[1], S.H. Marshall[1]
[1]Pontificia Universidad Católica de Valparaíso, Instituto de Biologia, Valparaiso, Chile

Backgrounds

Piscirickettsia salmonis is a gram-negative facultative intracellular bacterium, the etiological agent of Piscirickettsiosis, an infectious disease that affects the global salmon farming since the late 80's. The recent genome sequencing of different *P. salmonis* strains and natural isolates demonstrates that this agent contains a Dot/Icm Secretion System, homologue to those described for *Legionella pneumophila* and *Coxiella burnetii*. Based upon this information, we have demonstrated that *P.* destabilizes the actin cytoskeleton of the host and that is also able to inhibit the phagosome-lysosome fusion, putatively in a Dot/Icm dependent way.

Objectives

This work was focused in identifying and characterizing Dot/Icm substrates in *P. salmonis*, using *in silico* and subsequently molecular characterization of those putative virulent effectors.

Methods

The *in silico* identification was done using as reference Dot/Icm substrates described for *L. pneumophila* and *C. burnetii*, using as distinctive parameter the Dot/Icm secretion signal. Later, the identified ORFs in *P. salmonis* genome where analyzed in order to find conserved domains using the CDD search, SMART and Motif Search tools. Finally, the ORFs were cloned and expressed as recombinant proteins in order to determine their toxic effect over eukaryotic cells.

Conclusions

We have identified a number of Dot/Icm putative substrates in the *P. salmonis* genome. These proteins contains characteristic eukaryotic conserved domains: i) ankyrin repeats, ii) ADP-Ribosyl Transferase, iii) U-BOX, iv) PKc, v) ubiquitin ligase, vi) patatin, and vii) SEL-1. Expression of these proteins is in two different eukaryotic models: a fish macrophage cell line and in yeast, suggesting their putative role as virulence effectors.

FEMS7-0336
Pathogens / Pathogenicity

RIBOSWITCH REGULATION OF METHIONINE METABOLISM AND VITAMIN B12 UPTAKE IN MYCOBACTERIA – A ROLE IN PATHOGENESIS?

T. Kipkorir[1], *G. Mashabela*[1], *V. Mizrahi*[1], *D.F. Warner*[1]
[1]*University of Cape Town, Pathology and Institute of Infectious Disease and Molecular Medicine, Cape Town, South Africa*

Backgrounds

Mycobacterium tuberculosis (MTB) is thought to recognize fluctuations in specific environmental stimuli within the host, and to respond with corresponding alterations to gene expression and metabolism. Accordingly, the MTB genome contains a large number of regulatory elements which could contribute to this capacity. These include two vitamin B_{12} (B_{12}) riboswitches that have been identified upstream of *metE* – encoding a B_{12}-independent methionine synthase, and *ppe2* – the first gene in a putative operon with the B_{12} biosynthetic genes, *cobQ1* and *cobU*. We showed previously that disruption of the alternative, B_{12}-dependent methionine synthase, MetH, rendered MTB sensitive to B_{12}, presumably via methionine auxotrophy owing to B_{12} riboswitch-dependent suppression of MetE levels.

Objectives

Unlike MTB, MSM is a constitutive B_{12} producer under standard conditions *in vitro*. Therefore, we hypothesized that MetH would be essential in MSM, and that deletion of *metH* would be possible only by *metE* riboswitch inactivation or in a B_{12} deficient background.

Methods

Targeted knock-out of *metH* proved facile in a MSM Δ*cobK* mutant in which *de novo* B_{12} biosynthesis is disrupted. Curiously, however, the double Δ*metH* Δ*cobK* deletion mutant was not sensitive to exogenous B_{12}. Comparative genomic analyses identified additional B_{12} riboswitches controlling two separate BtuFCD-type B_{12} transporters that are present in MSM but not MTB.

Conclusions

These findings suggest that B_{12}-dependent metabolism in MSM is regulated both at the level of cofactor transport and enzymatic function, thus identifying B_{12} uptake as one potential point of departure in the evolution of the pathogenic MTB from the most recent common mycobacterial ancestor.

FEMS7-2805
Pathogens / Pathogenicity

CANDIDA ALBICANS IS ABLE TO ALTER ENTEROCYTE TIGHT JUNCTIONS THAT ENHANCES ITS TRANSLOCATION THROUGH THE GUT BARRIER

A. Loiselet[1,2], F. Bon[1], A. Ducreux[1], C. d'Enfert[3], B. Hube[4,5], F. Dalle[1,2]
[1]UMR A PAM Univ Bourgogne-Franche-Comte, UFR Sciences de Santé- Bât B3, Dijon, France
[2]Centre Hospitalier Universitaire, Service de Parasitologie Mycologie, Dijon, France
[3]Institut Pasteur- INRA, Unité Biologie et Pathogénicité Fongiques, Paris, France
[4]Leibniz Institute for Natural Product Research and Infection Biology—Hans Knoell Institute, Department of Microbial Pathogenicity Mechanisms, Jena, Germany
[5]University Hospital Jena, Center for Sepsis Control and Care, Jena, Germany

Backgrounds

C.albicans is commensal yeast of the mucosa in healthy humans that can be responsible for disseminated candidiasis, mainly originating from the gut. Deciphering the cellular and molecular mechanisms of the interaction of *C.albicans* with enterocytes is necessary to better understand the basis of commensalism and pathogenicity of the yeast and to improve the management of disseminated candidiasis. Different routes of tissue invasion by *C.albicans* are reported, partly due to differences in the structure of the epithelial layer. The intestinal epithelium consists in a monolayer of enterocytes where adjacent cells are jointed each other through cell junctions. Among those, tight junctions (TJ) ensure integrity and impermeability of the intestinal barrier, limiting invasion of the gut by *C.albicans.*

Objectives

The aim of our work is to investigate the impact of *C. albicans* interaction upon the integrity of the intestinal barrier.

Methods

Permeability of the intestinal barrier is investigated measuring the transepithelial electrical resistance of Caco-2 cell monolayers interacting with *C. albicans*. The organization of the TJ protein complex is studied by (i) monitoring their cellular localization using microscopy and (ii) their cellular distribution by using western blotting and Nano-HPLC. Molecules of the fungus responsible for these cellular alterations are currently under investigation.

Conclusions

Candida albicans is able to disorganize the TJ protein complex, altering the integrity of the intestinal barrier. As a consequence, *C. albicans* enhances its translocation through the gut barrier. Better understanding the first steps of *Candida* invasion will help to find target molecules to prevent its dissemination during severe candidiasis.

FEMS7-1514
Pathogens / Pathogenicity

SCREENING DISRUPTORS OF THE TOXIN–ANTITOXIN COMPLEX IN STAPHYLOCOCCUS AUREUS

Y. Ota[1], T. Miyamoto[1], A. Yokota[2], S. Tsuneda[1], N. Noda[2]
[1]Waseda University, Department of life science and medical bioscience, Tokyo, Japan
[2]National Institute of Advanced Industrial Science and Technology, Biomedical research institute, Tsukuba, Japan

Backgrounds

Toxin–antitoxin (TA) modules, which are implicated in microbial cell growth and survival, are widespread in prokaryotic cells. These modules comprise toxins, which are harmful to the host cell and antitoxins, which commonly neutralize the cognate toxins by forming these TA complexes. Therefore, TA complexes are considered promising targets for the development of novel antimicrobial agents to combat the rapid spread of antibiotic resistance in major human pathogens such as *Staphylococcus aureus*.

Objectives

Herein, we aimed to identify new antimicrobial peptides directly targeting the MazEF module, a typical TA module, in *S. aureus* by using a high-throughput screening method combining the phage display technique, massively parallel sequencing, and a continuous fluorometric assay.

Methods

We enriched candidate peptides with a high affinity for the MazF toxin by using phage display technology involving repetitive biopanning. The peptide amino acid sequences were comprehensively determined using massively parallel sequencing. Thereafter, we utilized a continuous fluorometric assay to assess the ability of the peptides to liberate the MazF toxin in the presence of the MazE antitoxin.

Conclusions

Using biopanning followed by massively parallel sequencing, we identified several peptides that specifically bind to the MazF toxin, and subsequently, using a continuous fluorometric assay, we revealed that some peptides overcome the inhibitory effect of the MazE antitoxin on the MazF toxin *in vitro*. Our method can be readily applied in the discovery of disruptors of other TA systems using direct binding mechanisms.

FEMS7-2139
Pathogens / Pathogenicity

COMPARATIVE GENOMICS OF THE WHOLE GENUS TENACIBACULUM REVEALS POTENTIAL NEW VIRULENCE FACTORS IN THE FISH PATHOGEN T. MARITIMUM

J.F. Bernardet[1], D. Pérez-Pascual[2], G. Michel[3], R. Larocque[3], E. Duchaud[1]
[1]Institut National de la Recherche Agronomique, Molecular Virology and Immunology, Jouy-en-Josas, France
[2]Institut Pasteur, Genetics of Biofilms, Paris, France
[3]Sorbonne Universités UMR 8227 CNRS-UPMC,
Integrative Biology of Marine Models- Station Biologique de Roscoff, Roscoff, France

Backgrounds

The genus *Tenacibaculum* currently encompasses 23 species with validly published names. While most *Tenacibaculum* species were isolated from marine animals, macro-algae, tidal flats and the open sea and likely play significant roles in organic matter recycling, a few other species are devastating marine fish pathogens. Among these, *T. maritimum* is a major concern to the marine fish farming industry worldwide.

Objectives

Despite the ecological and economic significance of *Tenacibaculum* species in marine environments, little is known about the molecular traits that define their contrasting lifestyles. Hence, this work aimed at identifying relevant features in relation to their ecological niches, including virulence determinants for the pathogenic species.

Methods

We carried out the complete genome sequencing of the type strains of all available *Tenacibaculum* species and performed extensive comparative genomic studies. These analyses revealed several potential virulence factors using *T. maritimum* as a reference species, among them a chondroitin AC lyase and a sphingomyelinase. The genes coding for these proteins were cloned in the pFO4 vector, and recombinant proteins were produced in soluble form in *Escherichia coli* BL21(DE3). The activity of the purified chondroitin AC lyase was assayed by measuring the increase in absorbance at 232 nm of the reaction products using chondroitin A and C sulfates as substrates. The specific enzymatic activity of the purified sphingomyelinase was evaluated using the fluorimetric Amplex Red Sphingomyelinase assay kit.

Conclusions

Our results yielded significant insights into the evolution of the genus *Tenacibaculum* and provided a unique opportunity to compare environmental and pathogenic species within the same genus. The genomic data encompassing the whole genus will serve as references to facilitate further studies, to devise new genotyping tools for epidemiological surveys, and to help identifying new enzymes potentially of interest, e.g. able to degrade the algal polysaccharides widely used in the food industry or involved in bacterial pathogenesis.

FEMS7-0417
Pathogens / Pathogenicity

SALMONELLA TYPHIMURIUM REQUIRES SOPB AND SIFA TO SURVIVE INTRACELLULARLY IN A SCV-LIKE COMPARTMENT IN DICTYOSTELIUM DISCOIDEUM

C. Valenzuela[1], *A. Sabag*[1], *Í.M. Urrutia*[1], *M. Varas*[2], *C.A. Santiviago*[1]
[1]*Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Santiago, Chile*
[2]*Universidad de Chile, Facultad de Ciencias, Santiago, Chile*

Backgrounds

Salmonella survival within eukaryotic cells is explained, in part, by its ability to establish an intracellular compartment known as the *Salmonella* containing vacuole (SCV). To this end, *Salmonella* exploits several effector proteins secreted by type-three secretion systems $T3SS_{SPI-1}$ and $T3SS_{SPI-2}$. We recently reported a role for effector proteins SopB and SifA in the survival of *Salmonella* Typhimurium in *Dictyostelium discoideum*, and the presence of wild-type bacteria in a vacuolar compartment within this amoeba.

Objectives

We evaluated whether the defect in intracellular survival of Δ*sopB* and Δ*sifA* mutants is explained by their inability to survive within SCV-like vacuoles.

Methods

We performed infections of *D. discoideum* expressing a VatM-GFP fusion using bacterial strains expressing the red fluorescent protein mCherry, and analyzed infected cells by confocal laser microscopy. VatM is a vacuolar ATPase used as marker of SCV membranes in other host cells.

Conclusions

According to our results, viable wild-type bacteria were detected laying within VatM-GFP positive vacuoles up to 6 h of infection. In the case of Δ*sopB* and Δ*sifA* mutants, only a few bacteria were detected within VatM-GFP positive vacuoles at 3 h of infection, and most *D. discoideum* cells contained remnants of killed bacteria. Altogether, our results suggest that *S.* Typhimurium resides in a SCV-like compartment in *D. discoideum* and requires effectors SopB and SifA to avoid degradation by this amoeba. We are currently characterizing the composition of the SCV-like compartment in *D. discoideum*.

Supported by FONDECYT grants 1140754 and 1171814, and CONICYT fellowships 21140615, 22140758 and 21150005.

FEMS7-0414
Pathogens / Pathogenicity - Part II

IGG PROTEASES IN CANINE MYCOPLASMAS

D.R. Brown[1], *D.L. Michaels*[1]
[1]*University of Florida, Infectious Diseases and Pathology, Gainesville Florida, USA*

Backgrounds

Certain streptococci encode endoproteases that cleave IgG at a specific epitope between the hinge and CH2 glycosylation sites. The IgG consequently loses all lymphocyte FcγR-mediated effector function and cannot activate complement. Orthologs differ among streptococcal species in their narrow selectivity for IgG of the hosts to which the bacteria are normally restricted, a feature thought to reflect fine-tuned adaptation to host immune defenses.

Objectives

We sought to identify streptococcal IgG protease orthologs in the genomes of mycoplasmas.

Methods

Mycoplasma canis, *Mycoplasma cynos*, *Mycoplasma opalescens*, *Mycoplasma spumans* and *Ureaplasma canigenitalium*, all normally found in dogs, encode the core sequence of the enzyme (GenBank Protein Cluster PCLA_442768). Its genomic context differs among species and some have N- or C-terminal fusions with other mycoplasmal proteins, which suggests origins by horizontal transfer possibly from *Streptococcus canis*. We compared non-synonymous to synonymous amino acid substitutions within the core sequence in streptococci and mycoplasmas but found little evidence that site-specific diversifying selection drives adaptation to the IgGs of different hosts. A predicted mannose and glucosamine ligand-binding exosite, possibly responsible for inital interactions with substrate IgG, was conserved in *M. canis*, *M. opalescens* and *U. canigenitalium* but degenerate in *M. cynos* and *M. spumans*. The IgG in normal dog serum was degraded in the signature pattern of streptococcal IgG proteases when incubated with *M. canis* but not with *M. cynos*.

Conclusions

We conclude that the IgG protease in certain mycoplasmas is evolutionarily, structurally and functionally related to streptococcal orthologs and may confer previously unrecognized immunosuppressive effects during mycoplasmosis of dogs.

FEMS7-2515
Pathogens / Pathogenicity - Part II

VIRULANCE AND RESISTANCE TRAITS IN BACTERIAL AND FUNGAL STRAINS ISOLATED FROM AIR IN THE HOSPITAL ENVIRONMENT

M.M. Mitache[1], C. Curutiu[2], A. Suwaraiy[2], M.C. Chifiriuc[2]
[1]Unviersity of Medicine Titu Maiorescu, preclinic, Bucharest, Romania
[2]University of Bucharest- Faculty of Biology, Microbiology, Bucharest, Romania

Backgrounds

Air microbiological analysis in the hospital environment allows to establish the risk of the air borne infections on one side, as well as the risk of wounds contamination and allergic processes induced by air microbes.

Objectives

The purpose of this study was to invetigate the virulence and resistance features in bacterial and fungal strains isolated from the hospital environment during the air microbiological control.

Methods

The study was carried in five general hospitals. The volumetric method using the MAS- 100 Eco equipment was used to harvest the desired air volume. The strains were isolated on Sabouraud + chloramphenichol and blood agar media, incubated at 37 Celsius degrees for 48 h. The isolated microorganisms have been identified using miniAPI galleries and investigated for antibiotic susceptibility profiles and for the production of cell-asociated (adherence to inert and cellular substratum, biofilm development) and soluble, enzymatic (hemolysins, lecithinase, lipase, caseinase, gelatinase, amylase, esculin hydrolysis) virulence factors, using phenotypic (disk diffusion method, selective media for enzymatic factors production) and PCR-based methods.

Conclusions

The isolated strains were identified as Staphylococcus xylosus, S. cohnii, S. xylosus, S. aureus, Enterococcus cloacae , Ent. aerogenes, Escherichia coli, Trichoderma viride, Penicillium sp., Altenaria sp., Fusarium roseum and Aspergillus niger.

FEMS7-0307
Pathogens / Pathogenicity - Part II

DISULFIDE BOND FORMATION IN GASTRIC PATHOGEN HELICOBACTER PYLORI: AN ACHILLAES' HEEL FOR SECRETION OF PRO-INFLAMMATORY VIRULENCE PROTEINS

C. Creuzenet[1], J. Lester[1], S. Kichler[1], H. Atanassov[2], C. Bodet[2], B. Oickle[1], S. Fairweather[1], M. Perivier[2], A. Oberc[1], J. Chahal[1], D. Ratnayake[1], C. Burucoa[2]
[1]Western University, Microbiology and Immunology, LONDON, Canada
[2]University Hospital, LITEC, Poitiers, France

Backgrounds

H. pylori causes gastritis, gastric ulcers and cancers but the mechanisms of virulence are not fully understood. It produces secreted proteins which may play a role in eliciting gastric inflammation, including the Helicobacter cysteine rich protein HcpE (HP0235) whose biological function is unknown.

Objectives

To investigate if HcpE is secreted by *H. pylori* and is involved in host / pathogen interactions, and identify components essential for its production.

Methods

This work uses anti-HcpE ELISA and Western blots, knockout mutagenesis, phenotypic analyses and biochemical assays.

Conclusions

We demonstrate that HcpE is secreted by many strains as a soluble protein and in association with outer membrane vesicles. We show that infected patients produce anti-HcpE antibodies, indicating in situ HcpE production. We show that HcpE comprises many disulfide bonds and identify DsbK (HP0231) as a folding factor necessary for HcpE production, and show that recombinant DsbK can refold unprocessed, reduced HcpE *in vitro*. This highlights the first biologically relevant substrate for DsbK. Furthermore, we show that DsbK has DiSulfide Bond (Dsb) forming activity on reduced lysozyme and has DsbA-like activity upon expression in *E. coli*, despite its similarity with DsbG. Also, we show a role of DsbK in redox homeostasis in *H. pylori*. Finally we show an important role for DsbK and HcpE in host-pathogen interactions, including murine gastric colonization and pro-inflammatory cytokine production in human gastric explants and in murine splenocytes. Both proteins will be investigated as therapeutic targets to treat *H. pylori* infections and prevent gastric ulcers and cancers.

FEMS7-2313
Pathogens / Pathogenicity - Part II

COMPARISON OF PHENOTYPIC TESTING AND COMPLETE GENOME SEQUENCE ANALYSIS FOR ANTIBIOTIC RESISTANCE GENES IN KLEBSIELLA PNEUMONIAE ISOLATES

N. García-González[1], L. Mejía-Castañeda[1,2], S. Sabater[3], B. Gomila[3], R. Moreno[4], F. González-Candelas[1,5]
[1]FISABIO-CSISP/Univ. Valencia, Joint Research Unit Infection and Public Health, Valencia, Spain
[2]Universidad San Francisco Quito, Dept. of Microbiology, Quito, Ecuador
[3]Hospital General Universitario de Castellón, Microbiology Service, Castellón, Spain
[4]Hospital General Universitario Castellón, Microbiology Service, Castellon, Spain
[5]CIBER in Epidemiology and Public Health, Genomics and Health, Valencia, Spain

Backgrounds

Antibiotic resistance is recognised as one of the major threats to public health and welfare. Increasing efforts are being invested to prevent and control the dissemination of antibiotic resistance mutations (AMRs) but rapid and accurate diagnosis are essential in this context. The advent of new high-throughput sequencing technologies opens new possibilities in this line, but their implementation at the clinical level needs a detailed evaluation in terms of sensitivity and precision but also in investments in time, training, and interpretation of the results.

Objectives

To compare the results of the usual phenotypic tests in a clinical microbiology laboratory with the detection of AMRs from the analysis of reads from complete genome sequences.

Methods

From a total of 1038 *K. pneumoniae* strains from 657 patients between March 2014 and February 2015, 111 showed ESBL resistance. Phenotypic tests were performed with ESBL+AmpCScreen Kit (ROSCO), KPC+MBL-confirmation kit (ROSCO) y PCR con Xpert Carba-R (Cepheid). Next generation sequencing was performed with Illumina NextSeq (2x150 paired-ends) using libraries prepared with Nextera. AMRs were identified in the sequencing reads using SRST2 with the ARG-annot (AntibioticResistance Gene-ANNOTation) database.

Conclusions

We obtained a high but not perfect match between phenotypically determined resistance and the inferred presence of AMR genes as inferred from the analysis of sequencing reads. Most strains (>90%) harbour at least two ESBL-producing genes, usually bla_{SHV} and $bla_{CTX\text{-}M}$. Phenotypic tests failed in detecting a $bla_{OXA\text{-}48}$ carrying strain. At present, WGS can complement but not completely replace phenotypic tests for AMR.

FEMS7-0551
Pathogens / Pathogenicity - Part II

ROLE OF REDUCTIVE AND NON-REDUCTIVE IRON ASSIMILATION ON THE VIRULENCE OF PENICILLIUM DIGITATUM

L. González-Candelas[1], C. Selma[1], A.R. Ballester[1]
[1]Instituto de Agroquímica y Tecnología de Alimentos IATA-CSIC, Food Biotechnology, Paterna, Spain

Backgrounds

Penicillium digitatum is the major postharvest pathogen of citrus fruit. It is a necrotrophic fungus with a very narrow host range that penetrates the fruit through injuries inflicted during harvesting and handling. In the last years, there has been an increasing interest in trying to find out the mechanisms used by this ascomycete to be such a successful pathogen. We have previously identified a set of fungal genes induced during pathogenesis. Gene ontology analysis of differentially expressed genes pointed to metal ion homeostasis as an important factor during pathogenesis. Iron is the most abundant metallic ion in nature and plays fundamental roles in living organisms. In aerobic environments, iron is mostly present as insoluble salts of Fe^{3+}. Fungi rely on two mechanisms for iron acquisition: a reductive iron assimilation (RIA) and a siderophore mediated assimilation.

Objectives

We aim to understand the role of these two iron assimilation pathways on the virulence of *P. digitatum*.

Methods

We have used *Agrobacterium*-mediated transformation to obtain *P. digitatum* knockout mutants lacking either *sidA*, the first gene in the siderophore biosynthesis pathway, or/and *ftrA*, encoding the permease involved in the RIA pathway, as well as on *hapX*, the major transcription factor that regulates the expression of iron dependent genes under iron-limited conditions.

Conclusions

We will present the characterization of these mutants and discuss their role on the virulence of *P. digitatum.*

FEMS7-1122
Pathogens / Pathogenicity - Part II

FUNCTIONAL SPECIALIZATION OF PECTOBACTERIA CELLS DURING COLONIZATION OF THE PLANT

V. Gorshkov[1,2], A. Daminova[1], R. Gubaev[1], O. Petrova[1], M. Ageeva[3], N. Gogoleva[1], E. Kovtunov[1], N. Tarasova[1], Y. Gogolev[1,2]
[1]Kazan Institute of Biochemistry and Biophysics - RAS, Molecular Biology, Kazan, Russia
[2]Kazan Federal University, Botany and Plant Physiology, Kazan, Russia
[3]Kazan Institute of Biochemistry and Biophysics - RAS, Microscopy, Kazan, Russia

Backgrounds

Although bacteria are unicellular organisms, the microbial population represents highly organized and developing structure with different morpho-physiological cell types being functional parts of this complex system. The research of the formation of differentiated microbial cells is in its infancy, especially taking into account integrated host/pathogen systems. Harmful phytopathogenic pectobacteria are considered to interact with plants by means of “brute force”. However, several prerequisites indicate that pectobacterias’ behavior *in planta* may be related to “stealth”.

Objectives

We tested if the colonization of the plant is coupled with dissociation of pectobacteria population and tried to obtain information on causes and consequences of the functional specialization of pectobacteria cells *in planta*.

Methods

We have “looked inside” infected plants using a variety of complementary methods, e.g. various types of microscopy and chromatography, gene cloning and their heterologous expression, mutagenesis, gene expression analyses (qPCR, RNA-Seq).

Conclusions

Our results show that different plant compartments form the specific signaling backgrounds that drive the behavior of microbes resulting in *in planta* dissociation of bacterial population. Herewith, various sub-populations display functional specialization (e.g. blocking the vessels, stress adaptation, tissue maceration, cell migration, etc). Novel biofilm-like structures – bacterial emboli were described and discussed to play significant roles in the formation of pathosystem. *In planta* dissociation of bacterial population is coupled with plant susceptible response that provides conditioning of the colonized compartment. Our observations are strengthened and deepened by the whole-transcriptome analysis of both plant and pathogen during their interactions. This study was supported by RSF (15-14-10022).

FEMS7-1031
Pathogens / Pathogenicity - Part II

C26 - A NOVEL COMPOUND INHIBITING THE EXPRESSION OF TYPE III SECRETION SYSTEM 1 OF SALMONELLA TYPHIMURIUM

I. Grin[1], A. Naini[2], S. Westerhausen[1], M. Riess[1], M. Brönstrup[2], S. Wagner[1]
[1]Interfaculty Institute of Microbiology and Infection Medicine Tübingen,
Research Group Prof. Samuel Wagner, Tübingen, Germany
[2]HZI Braunschweig, AG Prof. Mark Brönstrup, Braunschweig, Germany

Backgrounds

Antibiotic resistance of bacteria is a serious emerging threat and hence the search for new antibiotics is of high priority. A novel class of antibacterial compounds that block infection instead of killing bacteria is thought to exhibit a lower potential for the development of resistance because of a reduced selection pressure compared to conventional antibiotics. These antiinfectives target virulence mechanisms of pathogenic bacteria such as adhesion determinants or toxin delivery systems.

Type III secretion systems (T3SS) of Gram-negative bacteria, often enteropathogens, enable the injection of bacterial effector proteins into eukaryotic target cells to promote infection and colonization. Type III secretion systems are not only excellent targets for antiinfectives because the virulence of many pathogens depends on these systems but also because they are highly conserved and hence a single drug has the potential to act against a broad spectrum of enteropathogens.

Using a luciferase-based assay, we screened multiple proprietary as well as commercially available compound libraries for small molecule inhibitors of T3SS in our *Salmonella* model system. In this screen we could identify C26 - a novel selective inhibitor of *Salmonella* T3SS.**Objectives**

We want to identify the molecular target of C26 and elucidate the mode of action of the compound against the T3SS

Methods

Luciferase-based screening of small molecule inhibitors; mRNA sequencing; cytotoxicity assay;

Conclusions

We have identified C26, a strong inhibitor of the *Salmonella* type III secretion system 1.

The molecular target and mode of action of C26 are currently under investigation.

FEMS7-1985
Pathogens / Pathogenicity - Part II

ABSCISIC ACID EFFECT ON PECTOBACTERIUM ATROSEPTICUM VIRULENCE

R. Gubaev[1], V. Gorshkov[1,2], O. Petrova[1], O. Parfirova[2], S. Tatarkin[1], N. Gogoleva[1], Y. Gogolev[1,2]
[1]Kazan Institute of Biochemistry and Biophysics - RAS, Molecular Biology, Kazan, Russia
[2]Kazan Federal University, Botany and Plant Physiology, Kazan, Russia

Backgrounds

Mutual "eavesdropping" of partner's endogenous signals is a common phenomenon in plant-pathogen interactions. Virulence of *Pectobacterium atrosepticum* (*Pba*), causing plant soft rots, depends on water balance and ontogenetic processes of the host plants that are controlled by phytohormone abscisic acid (ABA). It is well-known that *Pba*-induced disease symptoms are not expressed under low humidity when the concentration of ABA is elevated in plants. However, still there is no information on ABA effect on pectobacteria physiology.

Objectives

The aim of the present study was to explore ABA effect on the *Pba* physiology, including the whole-transcriptome profile.

Methods

ABA effect on *Pba* was monitored by measuring bacterial growth, activity of the main virulence factors (extracellular pectate lyases), and by analyzing transcriptome profile by means of RNA-Seq. Additionally, ABA-signaling in plants was assessed in the course of *Pba*-caused infection.

Conclusions

ABA significantly reduced extracellular pectate lyase activity when added into bacterial cultures. This effect was not mediated by the influence on bacterial growth or post-transcriptional regulation of the enzymes. Gene expression analysis by means of qPCR and RNA-Seq revealed down-regulation of pectate lyase genes as well as some other virulence factors and regulatory proteins in the presence of ABA. This is in agreement with our data on repression of plant ABA-mediated system during acute infection and increased susceptibility of plants pretreated by inhibitor of ABA biosynthesis to *Pba*. Thus, through ABA content *Pba* is likely to “feel” the physiological status of the host and fine-tunes its virulence accordingly. This study was supported by RSF (15-14-10022).

FEMS7-0087
Pathogens / Pathogenicity - Part II

PIECING TOGETHER YSCX FUNCTION: THE CRITICAL PROTEIN IN YERSINIA TYPE III SECRETION SYSTEM

J.M. Gurung[1], A.A.A. Amer[1], S. Chen[2], M.S. Francis[1]
[1]Umeå University, Department of Molecular Biology, Umeå, Sweden
[2]Chinese Academy of Sciences, Wuhan Institute of Virology, Wuhan, China

Backgrounds

Yersinia bacteria utilize a type III secretion system (T3SS) to target effector toxins into the host cells which promote mutualistic or pathogenic associations. T3SS's are comprised of ~25 structural proteins many of whose functions remain obscure. In *Yersinia*, YscX and YscY are two poorly characterised proteins. However, the absence of Ysc-Yop T3SS in *yscX* or *yscY* null mutant suggests that YscX and YscY are important Ysc-Yop T3SS constituents. This may arise from a need to form a binary interaction involving YscX-YscY or a ternary interaction with the inner membrane export component YscV.

Objectives

Study the contribution of YscX secretion towards T3S function.

Methods

To understand the role of YscX, we initially investigated the functional interchangeability between genetically conserved members of YscX-YscY protein families from diverse bacteria. Our study suggested that the function of YscX might be specific to *Yersinia* despite reciprocal binding to YscY and YscV family members. To further dissect the uniqueness of YscX function, we scrutinized the role of YscX N-terminus in secretion of itself and other T3S proteins. Site-directed mutagenesis and defined domain swapping revealed YscX N-terminus to be central for Ysc-Yop activity as it prevented YscF needle assembly and abolished T3SS activity. Critically, the inability of these YscX variants to impede T3SS was not due to the disruption of their secretion ability or destabilization of binary and ternary interactions.

Conclusions

Hence, the YscX N-terminus is dual functional; on the one hand it is a secretion recognition motif and on the other, a novel structural component necessary for the correct assembly of the Ysc-Yop T3SS.

FEMS7-0304
Pathogens / Pathogenicity - Part II

VIRULENCE POTENTIAL OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI ISOLATED FROM THE ANIMAL RESERVOIR IN BRAZIL

B.E.C. Guth[1], C.F. Freire[1], C. Matheus-Guimaraes[1], E.M. Gonçalves[1], L.F. Dos Santos[1]
[1]Universidade Federal de São Paulo, Microbiology- Immunology- and Parasitology, São Paulo, Brazil

Backgrounds

Shiga toxin-producing *Escherichia coli* (STEC) are food-borne pathogens being ruminant animals their main natural reservoir. Several non-O157 STEC serotypes unable to cause attaching and effacing lesion (A/E) have been implicated as agents of serious human diseases.

Objectives

Evaluate the distribution of *stx* subtypes and accessory virulence genes in non-O157 STEC isolates devoid of A/E from the animal reservoir in order to identify their pathogenic potential.

Methods

Gene sequences related to Stx1 and Stx2 subtypes, autotransporter proteins (EspP, EspI, EpeA, Sab), adhesins (Saa), toxins (EhxA), iron-repressible protein (Irp2) and metalloprotease (StcE) were sought by PCR in 125 STEC strains isolated from sheep, goat, buffalo and cattle. The strain phylogroup was also determined.

Conclusions

STEC isolates most frequently harbor *stx*2 (52%) and *stx1stx2* (38%), being subtypes *stx1a* (57%), *stx1c* (47%), *stx2a* (67%), and *stx2d* (38%) those most frequently identified. Distribution of subtypes varied according to the reservoir**:** *stx1a* prevailed in cattle and buffalo isolates, *stx1c* predominate in sheep and goat, *stx2a* in cattle, and *stx2d* prevailed in goat and buffalo.The *saa*, *espP*, *ehxA*, *espI, epeA*, *sab*, *irp2* and *stcE* genes were identified in 89%, 58%, 55%, 43%, 30%, 13%, 11% and 9% of the STEC isolates, respectively, and differences were also observed depending on the reservoir. *espP* and *ehxA* molecular subtyping showed that variants C (90%) and A (100%) prevailed, respectively. B1 was the predominant phylogroup identified. Overall STEC isolates from cattle most frequently carry the *stx2* subtypes related to human infections, and harbor virulence factors at higher frequencies.

FEMS7-2763
Pathogens / Pathogenicity - Part II

DEVELOPMENT OF CHITOSAN/HEPARIN NANOPARTICLES FOR TARGETED ANTIMALARIAL DRUG DELIVERY

L. Gutiérrez Chamorro[1], *E. Lantero*[2], *X. Fernández-Busquets*[3]
[1]*ISGlobal- Hospital Clinic de Barcelona- Universitat de Barcelona, Nanomalaria, Barcelona, Spain*
[2]*Institute for Bioengineering of Catalonia IBEC, Nanomalaria, Barcelona, Spain*
[3]*Institute for Bioengineering of Catalonia IBEC- ISGlobal- Universitat de Barcelona, Nanomalaria, Barcelona, Spain*

Backgrounds

Malaria, a life-threatening disease caused by parasites of the genus *Plasmodium*, is still the most prevalent parasitic disease in the world. Keeping in mind that an effective vaccine against malaria is still under development, chemotherapy is one of the most important strategies to reduce its associated morbidity and mortality. However, the emergence of drug-resistant malaria parasites, combined with the non-specific targeting of current chemotherapies to intracellular parasites, highlights the necessity of new therapeutic and drug delivery approaches, like novel antimalarial drugs and improved methods for their efficient administration. In this context, nanomedicine enables numerous potential opportunities for malaria treatment such as nanovector-based drug delivery strategies. Polysaccharides, in particular chitosan -a deacetylated chitin derivative-, have gained increasing attention because of their numerous advantages. In addition, data from single-molecule force spectroscopy and fluorescence microscopy have shown binding specificity of heparin to *Plasmodium*-infected red blood cells *vs*. non-parasitized erythrocytes.

Objectives

In this project, we aim to establish a method for the synthesis of antimalarial drug-loaded chitosan/heparin nanoparticles and evaluate their specific antimalarial activity.

Methods

For this purpose, nanoparticles are prepared by the ionic gelation method. In order to characterize them, we use transmission electron microscopy and dynamic light scattering. Finally, growth inhibition assays in *Plasmodium falciparum* cultures are performed in order to determine antimalarial activity and targeting of the nanoparticles to parasitized red blood cells.

Conclusions

In conclusion, we explore a new cost-efficient approach for the treatment of malaria based on a nanomedical strategy that could provide a targeted delivery contributing to reducing resistance evolution.

FEMS7-2429
Pathogens / Pathogenicity - Part II

GALLERIA MELLONELLA AS IN VIVO MODEL FOR THE STUDY OF THE PATHOGENICITY OF ADHERENT INVASIVE ESCHERICHIA COLI (AIEC)

D. Gutiérrez[1], C. Castaneda-Alvarez[2], G. Vidal[2], E. Aballay[2], F. Del Canto[2], R. Vidal[1]
[1]Universidad de Chile, Department of Microbiology and Mycology, 8380453, Chile
[2]Universidad de Chile, Department of plant health, 8380453, Chile

Backgrounds

Adherent Invasive *Escherichia coli* (AIEC) are defined by the in vitro capacity of adhere and invade intestinal epithelial cells and also survive and replicate within macrophages. AIEC has been frequently isolated from patients with Crohn's disease, a chronic inflammatory bowel disease.

Animal models, mainly mice, represent an indispensable tool for understand the physiological and mechanistic human-microbial interaction. Other in vivo models such as *Dictyostelium discoideum*, *Caenorhabditis elegans*, *Drosophila melanogaster* and *Danio rerio* have been implemented. However, none of these organisms is possible to maintain at 37°C, temperature at which most bacterial pathogens express their virulence factors. *Galleria mellonella* (wax moth) is a simple invertebrate that recently has been used as a model for the study of human pathogens and the early results reported so far suggest a correlation with the pathogenicity displayed in murine models.

Objectives

To determine if *Galleria mellonella* larvae could be used as a model to study AIEC pathogenesis.

Methods

Larvae were injected with AIEC strains and non-pathogenic *E. coli* HB101 (10^3-10^7 UFC/10 µL). Survivors were everyday recorded for five days.

Conclusions

G. mellonella are susceptible to AIEC infection, and larval mortality is dose dependent (100% lethality with 10^6 CFU / larvae of AIEC 4C01, LD_{50} = 2.1 x 10^4). In contrast, the inoculum with non-pathogenic *E. coli* HB101 strain didn't cause death at a dose of 10^6 CFU / larvae (100% survival). This is the first report in which *G. mellonella* is proposed as an *in vivo* model for future AIEC infection studies.

FEMS7-2388
Pathogens / Pathogenicity - Part II

IMPLICATION OF MOBILE GENETIC ELEMENTS IN THE VIRULENCE OF STAPHYLOCOCCUS AUREUS STRAINS FROM CYSTIC FIBROSIS PATIENTS

K. Guzman[1], M. Cervera-Alamar[1], M. Žiemytė[1], A. García-Ortega[2], M. Martí Jimenez[3], R.M. del Campo Moreno[4], A. Solé Jover[2], M.A. Tormo Mas[1]
[1]HOSPITAL LA FE, Several Infeccion Group, VALENCIA, Spain
[2]HOSPITAL LA FE, Adult Cystic Fibrosis Unit- Microbiology Service, VALENCIA, Spain
[3]Catholic University of Valencia, Experimental Sciences and Mathematics Department, Valencia, Spain
[4]University Hospital Ramón y Cajal- and Instituto Ramón y Cajal de Investigación Sanitaria, Biology and Evolution of Microorganisms Group- Microbiology Department, Madrid, Spain

Backgrounds

Staphylococcus aureus is one common pathogen that colonize the lung of cystic fibrosis (CF) patients. *S. aureus* versatility is due to its ability to persist and multiply in different environments and to produce a several virulence factors, most of which are encoded by mobile genetic elements (MGE).

Objectives

The aim of this work was to molecularly characterize a collection of 200 *S. aureus* isolates obtained from 118 CF-patients attended in a single Unit, in Valencia, Spain.

Methods

Antibiotic susceptibility was determined by disk diffusion test and presence of *mec*A gene was corroborated by PCR. Multi Locus Sequence Typing (MLST) and Pulsed-Field Gel Electrophoresis (PFGE) techniques were performed in all isolates, and presence of Mobile-Genetic-Elements (MGEs) was determined by integrase PCR, Southern Blot and Screening Lysates.

Conclusions

Methicillin-resistance was observed in 21.5% of the isolates. Elevated adherence capability was demonstrated in about 60% of them. High genetic diversity was found including 47 STs, some of them non-previously described. Transmission between patients was not detected. Curiously, some discrepancies between MLST and PFGE fingerprinting were observed, probably in relation with MGE variations. All isolates carried at least one phage, and 85.5% of them were able to lysate the bacterial cultures. SaPI integrases detected in 49.5% of isolates. Unexpectedly, 28% of SaPIs were induced by its own endogenous phages.

CF-*S. aureus* present high rate of MGE that might be involved in pathogenicity and virulence activation and spread. Some discrepancies between the MLST and PFGE techniques were detected suggesting ecological adaptation to adverse conditions.

FEMS7-0236
Pathogens / Pathogenicity - Part II

GROEL-MEDIATED INDUCTION OF PTX3 IS UNDER THE CONTROL OF NF-KB AND MIRNA-9

Y. Lee[1], J. Jeon[1], H. Shin[2], U.H. Ha[1]
[1]Korea University, Biotechnology & Bioinformatics, Sejong, Republic of Korea
[2]Medytox, DS Part, Cheongju, Republic of Korea

Backgrounds

PTX3 has been recognized as an important soluble form of pattern recognition receptor, which plays a role in the clearance of microbial pathogens including *Pseudomonas aeruginosa*. Recently, we found that GroEL released from *P. aeruginosa* could act as a potent inducer for the expression of *PTX3*.

Objectives

The related mechanisms promoting the production of PTX3 remain unclear.

Methods

We measured mRNA and protein levels of PTX3 by applying real-time Q-PCR and ELISA. Signaling pathways were monitored by using knockout cell lines as well as transfection of dominant negative and miRNA mimics.

Conclusions

To verify the inducing effect of purified recombinant GroEL, we used Triton X-114, known as an effective reagent to remove lipopolysaccharide (LPS). Triton X-114 treatment thoroughly removed LPS, which could be contaminated during purification, and the resulting recombinant GroEL clearly induced the expression of *PTX3*. In addition, the induction was completely diminished by pretreatment of proteinase K, further supporting the action of recombinant protein-mediated induction. The induction of *PTX3* expression in response to GroEL was shown to be under the control of TLR4/NF-kB signaling pathways. In addition, GroEL treatment decreased the expression of miRNA-9, which targets *PTX3* transcripts, and the resulting miRNA-9 reduction was also under the control of TLR4. *P. aeruginosa*-derived GroEL is able to increase the production of PTX3 through the signaling pathways of TLR4-NF-kB and miRNA-9.

FEMS7-0237
Pathogens / Pathogenicity - Part II

T3SS EFFECTOR EXOY REDUCES THE EXPRESSIONS OF INFLAMMATORY CYTOKINES THROUGH DELAYING THE ACTIVATION OF NF-KB

J. Jeon[1], Y. Kim[2], H. Shin[3], U.H. Ha[1]
[1]Korea University, Biotechnology & Bioinformatics, Sejong, Republic of Korea
[2]KRIBB, Cell Factory Research Center, Daejeon, Republic of Korea
[3]Medytox, DS Part, Cheongju, Republic of Korea

Backgrounds

Pseudomonas aeruginosa possesses diverse virulence factors that are essential in the pathogenesis of bacteria to modulate host immune responses. Among them, type III secreted effectors, which are directly translocated into the cytoplasm of infected host cells through Type III Secretion System, have obtained much attention for their effects on manipulation of host cell function and viability.

Objectives

Little is known about the impact of one of effectors, ExoY, on the regulation of inflammatory cytokine expressions.

Methods

We analyzed effects of translocated ExoY in the expressions of pro-inflammatory cytokines at the levels of mRNA and protein.

Conclusions

We explored the impact of ExoY on the expressions of inflammatory cytokines including IL-1a, IL-1b, IL-8 and TNF-a. The expressions were clearly enhanced in response to *exoY* deletion mutants whereas the expressions were reduced by *exoY*-complementation. Furthermore, transfection of *exoY* into host cells was sufficient to reduce the expression of IL-1b stimulated by the treatment of not only *exoY* deletion mutants but also phorbol 12-myristate 13-acetate (PMA), a well-known inducer of inflammatory cytokines. The resulting suppression was found to be mediated by delaying the activation of NF-kB pathway. This study demonstrates that ExoY derived from *P. aeruginosa* can initiate anti-inflammatory responses by reducing the expressions of inflammatory cytokines through delaying the activation of NF-kB pathways.

FEMS7-1036
Pathogens / Pathogenicity - Part II

CITRUS TRISTEZA VIRUS CHANGES THE BIOCHEMICAL AND PHYSIOLOGICAL STATUS OF INFECTED MEXICAN LIME PLANTS

K. Hančević[1], B. Urlić[1], I. Pasković[2], T. Radić[1]
[1]Institute for Adriatic Crops and Karst Reclamation, Department of Plant Sciences, Split, Croatia
[2]Institute of Agriculture and Tourism, Department of Agriculture and Nutrition, Poreč, Croatia

Backgrounds

Reactions that occur when the plant is subjected to *Citrus tristeza virus* infection often results in triggering numerous defence mechanisms to fight the infection. The reactions vary regarding virus strain, host genotype, time exposure to the infection and environmental conditions. To date no study has examined the consequence of ten-years exposure to CTV infection on biochemical and physiological status of susceptible Mexican lime plants.

Objectives

To understand such kind of infection, changes in nutrient status, total proteins, enzyme activities involved in scavenging stressed conditions, photosynthetic and transpiration rates, membrane permeability and relative water content were analysed in lime plants inoculated with different CTV strains and healthy plants.

Methods

Enzymes and minerals were assayed following spectrophotometric methods. Net photosynthesis and stomatal conductance were measure by open gas exchange system (Li-COR 6400) and membrane permeability by electrical conductivity.

Conclusions

The activity of superoxide dismutase and polyphenol oxidase significantly decreased in the infected leaves, as well as membrane permeability. Macro- and micro-nutrient statuses were significantly changed: concentrations of leaf nitrogen, zinc, magnesium and iron were elevated but potassium concentration depressed in relation to non-infected control leaves. As shown in this study, clear physiological changes were found between infected and non-infected plants but none between the plants inoculated with different CTV strains. Data suggest that the most of the defence mechanism investigated here were supressed due to the continuous and long-term pressure of CTV biotic stress.

FEMS7-2517
Pathogens / Pathogenicity - Part II

IN VITRO ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF PIMPINELLA ANISUM ON CORYNEBACTERIUM BOVIS AND LISTERIA MONOCYTOGENES

A. Hashempour[1], S. Seyed gholizade[1], E. Mir ahmadi[1], M.H. Ebrahimi jam[2], A. Khorsandian[1]
[1]Urmia Branch -Islamic Azad Univercity, microbiology, Urmia, Iran
[2]Razi institute, virology vaccine, karaj, Iran

Backgrounds

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans as well as valuable components of seasonings, beverages, cosmetics, dyes, and medicines.Aromatic plants and essential oils extracted from these plants are becoming more important due to their antimicrobial effects and the stimulating effect on animal digestive systems. Aromatic plants have been used traditionally in the therapy of some diseases for a long time in the world.

Objectives

Therefore, the present investigation was undertaken to evaluate antibacterial activity of *Pimpinella anisum* seeds against *Corynebacterium bovis* and *Listeria monocytogenes***.**

Methods

The antimicrobial activity of essential oil was determined by the disc diffusion method. Susceptibility tests were performed by the disc diffusion method of Bauer et al with Mueller Hinton agar (W.Lawrence Drew et al 1972). Zones of inhibition were measured after 24 hr and again after 48 hr of incubation at 35-40 ˚C. Studies were also carried out by incubating the plates at 35 - 40 ˚C (Sadeghi Zali M.H. et al 2011).

Conclusions

The antibacterial activity of *Pimpinella anisum* essential oils was assayed in vitro by agar disc diffusion method against *Corynebacterium bovis* and *Listeria monocytogenes*. Table 2 summarizes the microbial growth of essential oils of the *Pimpinella anisum*. The *Pimpinella anisum* essential oil showed maximum antibacterial activity against Gram-positive bacteria.

FEMS7-3135
Pathogens / Pathogenicity - Part II

THE EFFECT OF IPN VIRUS IN THE PROGRESSION OF IMMUME SYSTEM OF SALMO SALAR

D. Haussmann[1,2,3], J. Blanco[1], F. Lagos[1,3], I. Adolfo[1], F. Jaime[1,3]
[1]Instituto de Bioquímica y Microbiología, Laboratorio de Biología Molecular de Peces, Valdivia, Chile
[2]Departamento de Ciencias Básicas- Facultad de Ciencias, Universidad Santo Tomas, Valdivia, Chile
[3]Centro FONDAP, Interdisciplinary Center for Aquaculture Research INCAR., Valdivia, Chile

Backgrounds

Backgrounds: The infectious pancreatic necrosis is a multi-systemic disease that affects salmonid species, causing millions of losses in the world aquaculture industry. It is caused by the virus IPN (IPNv) and is classified within the genus aquabirnavirus, consisting of a bi-segmented double-stranded RNA genome.

The disease together with the virus triggers the immune response, a complex defense mechanism that is divided into innate and adaptive immunity. In fish, innate immunity is the most important in defending against pathogens; immunity is modulated by molecules called cytokines, non-type cytokine proteins and membrane receptors.

Objectives

Objectives and Methods: The objective was to determine if IPNv modifies the expression of transcripts related to the immune system (CD8 + α, NCCRP-1, IL-1β, IL-12, TNF-α, IRF-1, IFNγ, IFNα, IFNβ and Mx).

Methods

Using propagation and viral titration techniques, kinetics of infection in primary culture of *Salmo salar* head kidney and qPCR.

Conclusions

Results and Conclusions: It was determined that, IPNv modifies the expression of the transcripts studied, increasing both the expression of IRF-1, IFNβ, CD8 + α, NCCRP-1, IL-1β, IL-12, and increasing gene expression in late times as Mx and IFNγ. It is observed that between 4 and 16 hours post-infection, the expression of most transcripts is low.

In conclusion, the virus IPNv, inhibits the expression of certain markers in early stages of infection, however, the virus manages to persist during the course of the infection.

This study is important because it will contribute to establish an early and effective diagnosis of the fish a viral infection.

FEMS7-2963
Pathogens / Pathogenicity - Part II

OPPORTUNISTIC INFECTIONS IN HIV-INFECTED PATIENTS: HAART ERA DID NOT LEAD TO A CHANGE IN THEIR SPECTRA IN PATIENTS EXAMINED POSTMORTEM

P. Heneberg[1], *M.K. Powell*[1]
[1]*Charles University, Third Faculty of Medicine, Prague, Czech Republic*

Backgrounds

AIDS-related mortality has changed dramatically with the onset of highly active antiretroviral therapy (HAART), which has even allowed compensated HIV-infected patients to withdraw from secondary therapy directed against opportunistic pathogens.

Objectives

We hypothesized that the pathogens frequency and spectrumchanges found in HIV-infected patients examined postmortem did not recapitulate the changes found previously in HIV-infected patients examined antemortemin both the pre- and post-HAART eras.

Methods

We performed autopsies on 124 HIV-infected patients who died from AIDS or other co-morbidities in the Czech Republic between 1985 and 2014. The pathological findings were retrieved from the full postmortem examinations and autopsy records.

Conclusions

We collected a total of 502 host-pathogen records covering 82 pathogen species, a spectrum that did not change according to patients' therapy or since the onset of the epidemics, which can probably be explained by the fact that even recently deceased patients were usually decompensated (in 95% of the cases, the last available CD4+ cell count was falling below 200 cells*µl-1) regardless of the treatment they received. The newly identified pathogen taxa in HIV-infected patients included *Acinetobacter calcoaceticus*, *Aerococcus viridans* and *Escherichia hermannii*.We observed a very limited overlap in both the spectra and frequencies of the pathogen species found postmortem in HIV-infected patients in Europe, the USA and Latin America. The shifts documented previously in compensated HIV-infected patients examined antemortem in the post-HAART era are not recapitulated in mostly decompensated HIV-infected patients examined postmortem.

FEMS7-3190
Pathogens / Pathogenicity - Part II

ANTIBACTERIAL ACTIVITY OF THE PLANT-DERIVED ISOTHIOCYANATES IS CONNECTED WITH INDUCTION OF THE STRINGENT RESPONSE

A. Herman-Antosiewicz[1], *M. Maciąg-Dorszyńska*[2], *D. Nowicki*[2], *A. Szalewska-Pałasz*[2]
[1]*University of Gdansk, Medical Biology and Genetics, Gdańsk, Poland*
[2]*University of Gdansk, Bacterial Molecular Genetics, Gdańsk, Poland*

Backgrounds

Isothiocyanates (ITC) are hydrolysis products of glucosinolates, secondary metabolites abundant in Brassicaceae plants. They are known from their chemopreventive and anticancer potential which relies on modulation of activity of enzymes responsible for carcinogen activation and detoxification in healthy cells as well as on inhibition of cell cycle and induction of apoptosis in cancer cells. ITC possess anti-inflammatory, immunomodulatory and anti-oxidant properties, and showed cardioprotective and neuroprotective activities in animal models of stroke, ischemia/reperfusion, neurodegeneration and spinal cord injury. Moreover, it has been shown that ITC have antimicrobial activity; however, the underlying mechanisms are still underexplored. Recently, it has been reported that some ITC induce stringent response in enterohemorrhagic *Escherichia coli*.

Objectives

We evaluated whether the activity of aliphatic (SFN) and aromatic isthiocyanate (PEITC) toward different Gram (-) and Gram (+) bacteria correlates with the stringent response induction.

Methods

We studied the effect of SFN (1- isothiocyanato-4- (methylsulfinyl)-butane) and PEITC (phenethyl isothiocyanate) on growth of different bacteria species: model bacterium, *Escherichia coli*, and opportunistic human pathogens, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* and their clinical isolates. The minimum inhibitory concentration as well as impact of ITC on RNA synthesis and ppGpp production were evaluated.

Conclusions

ITC were more potent toward Gram-positive than Gram-negative bacteria, however response of individual bacteria species varied. Moreover, sensitivity of bacteria to ITC was correlated with induction of the stringent response.

FEMS7-0285
Pathogens / Pathogenicity - Part II

INVOLVEMENT OF RALFURANONES IN THE QUORUM SENSING SIGNALING PATHWAY AND VIRULENCE OF RALSTONIA SOLANACEARUM STRAIN OE1-1

Y. Hikichi[1], Y. Mori[1], K. Hayashi[1], A. Kiba[1], K. Ohnishi[2], K. Kai[3]
[1]Kochi University, Laboratory of Plant Pathology & Biotechnology, Nankoku, Japan
[2]Kochi University, Research Institute of Molecular Genetics, Nankoku, Japan
[3]Osaka Prefecture University, Graduate School of Life and Environmental Sciences, Sakai, Japan

Backgrounds

The soil-borne plant pathogenic *Ralstonia solanacearum* strain OE1-1 produces and secretes methyl 3-hydroxymyristate (3-OH MAME) as a quorum sensing (QS) signal, which contributes to its virulence. A global virulence regulator, PhcA, functioning through the QS system positively regulates the expression of *ralA*, which encodes furanone synthase, to produce aryl-furanone secondary metabolites, ralfuranones. Ralfuranones contribute to full virulence of vstrain OE1-1.

Objectives

We investigated the functions of ralfuranones on OE1-1 virulence.

Methods

We analysed *R. solanacearum* transcriptome data generated by RNA sequencing technology. A ralfuranone-deficient mutant (Δ*ralA*)expressed *phcB*, which is associated with 3-OH MAME production, and *phcA* at levels similar to those in strain OE1-1. Additionally, Δ*ralA* exhibited downregulated expression of more than 90% of the QS-positively regulated genes, and upregulated expression of more than 75% of the QS-negatively regulated genes. These results suggest that ralfuranones affect the QS feedback loop. Ralfuranone supplementation restored the ability of Δ*ralA* cells to aggregate. Additionally,ralfuranones A and B restored the swimming motility of Δ*ralA* to wild-type levels. However, the application of exogenous ralfuranones did not affect the production of the major exopolysaccharide, EPS I, in Δ*ralA.* Quantitative real-time PCR assays revealed that the deletion of *ralA* results in downregulated expression of *vsrAD* and *vsrBC*, which encode a sensor kinase and a response regulator, respectively, in the two-component regulatory systems that influence EPS I production. The application of ralfuranone B restored the expression of these two genes.

Conclusions

Overall, our findings indicate that integrated signalling *via* ralfuranones influences the QS and virulence of *R. solanacearum*.

FEMS7-3083
Pathogens / Pathogenicity - Part II

HOW TOXIN-ANTITOXIN SYSTEMS CONFER VIRULENCE PLASMID STABILITY IN SHIGELLA SPP

G. McVicker[1], S. Hollingshead[2], C. Tang[2]
[1]Nottingham Trent University, College of Science and Technology- School of Science & Technology, Nottingham, United Kingdom
[2]University of Oxford, Sir William Dunn School of Pathology, Oxford, United Kingdom

Backgrounds

Pathogenic *Shigella spp*. are the leading cause of bacterial dysentery, with *Shigella flexneri* and *Shigella sonnei* accounting for around 90% of cases worldwide. *S. flexneri* predominantly causes disease in low-income countries, whilst *S. sonnei* prevails in wealthy countries. Both species contain a large virulence plasmid, pINV, which is essential for pathogenesis. *S. sonnei* pINV is less stable than *S. flexneri* pINV. Both plasmids contain a number of toxin–antitoxin systems, which are required for plasmid maintenance. Differences between the toxin-antitoxin systems harboured on the *S. sonnei* and *S. flexneri* virulence plasmids are responsible for their differential stability.

Objectives

Here, we report the isolation of a *S. sonnei* virulence plasmid with greatly increased stability at physiologically relevant temperatures. Our objective was to identify the genetic and biochemical basis for increased plasmid stability.

Methods

Close inspection of the toxin-antitoxin systems on pINV, revealed heightened plasmid stability is caused by a mutation affecting an antitoxin. We subsequently identified the biological function of this antitoxin mutation *in vitro*.

Conclusions

Using biochemical and structural analyses we determined the mechanism by which increased plasmid stability is conferred and thus how this strain has the potential for heightened pathogenesis and prolonged infection.

FEMS7-0656
Pathogens / Pathogenicity - Part II

LOW-LEVEL ANTIMICROBIAL RESISTANCE OF CRONOBACTER SPP. STRAINS ISOLATED FROM CLINICAL AND ENVIRONMENTAL MATERIAL

O. Holý[1], M. Röderová[2], J. Petrželová[2], J. Vlčková[1]
[1]Faculty of Medicine and Dentistry- Palacký University Olomouc, Preventive Medicne, Olomouc, Czech Republic
[2]Faculty of Medicine and Dentistry- Palacký University Olomouc, Microbiology, Olomouc, Czech Republic

Backgrounds

Cronobacter spp. is Gram-negative, facultative-anaerobic, nonspore-forming, motile bacteria belonging to the family *Enterobacteriaceae*. Infants up to two month of age, premature with low birth weight or immunocompromised newborns are at the highest risk for infection. *Cronobacter* spp. has been also recognized as the causative agents of various infections in elderly adults suffering from serious underlying disease or malignancy.

Objectives

The aim of the study was to estabilish antibiotics susceptibility of *Cronobacter* spp. strains isolated from clinical as well as environmental material.

Methods

Altogether, seventy-four *Cronobacter* spp. strains were used in this study (fifty clinical and twenty-four environmental strains). The strains had been collected during a survey of *Cronobacter* carriage by patients from two hospitals, during a 6-years period from May 2007 to August 2013. All *Cronobacter* spp. strains were tested for susceptibility against 17 types of antibiotics. The susceptibility to antibiotics was assessed by a standard microdilution method according to the European Committee on Antimicrobial Susceptibility Testing criteria. ESBL genes (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$) were tested by PCR method.

Conclusions

Susceptibility tests revealed that the isolates were susceptible to all antibiotics. No resistant strain was identified. Simultaneously, all strains were negative for *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes beta-lactamases. So far, antibiotics resistance is not a serious threat among *Cronobacter* spp. strains. However, the extensive use of antimicrobials in agriculture and health-care facilities has led to the emergence of resistant bacterial strains. Antimicrobial resistances are a public health concern, because it may cause failure of conventional treatment, resulting in prolonged illness and a higher risk of mortality.

The study was supported by the "Research Support Foundation Vaduz - Comprehensive Study at the Issue of Oncological Diseases" and IGA_LF_UP_2016_022.

FEMS7-3164
Pathogens / Pathogenicity - Part II

DETECTION AND CHARACTERIZATION OF ENTEROBACTERIA PRODUCING BETA-LACTAMASES FROM PATIENTS WITH LEUKAEMIA

O. Holý[1], M. Röderová[2], J. Vlčková[1], P. Jana[2], D. Horáková[1]
[1]Faculty of Medicine and Dentistry- Palacký University Olomouc, Preventive Medicne, Olomouc, Czech Republic
[2]Faculty of Medicine and Dentistry- Palacký University Olomouc, Microbiology, Olomouc, Czech Republic

Backgrounds

Acute leukaemia (AL) is a heterogeneous group of malignant diseases of haematopoiesis. AL is divided into: acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL).

Objectives

The aim of this study was to carry out active surveillance of antibiotics resistance among enterobacteria isolated from patients with acute leukaemia.

Methods

101 clinical isolates were obtained from the 95 patients from September 2015 to August 2016. Out of these, 26 patients suffered from ALL and 69 patients from AML. Bacterial isolates were identified using MALDI-TOF MS. This method was also used for strains typing and dendrograms were created. The susceptibility to antibiotics was assessed by a standard microdilution method. All isolates were tested for beta-lactamase genes (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$) by PCR method.

Conclusions

Overall, the most common material was throat swab (n=35), stool (n=29) and urine (n=18). Most often, *K. pneumoniae* was present (n=31) and *K. oxytoca* (n=10), followed by *E. coli* (n=22). High clonality among strains was observed. Genes encoding production of TEM, SHV and CTX-M types of beta-lactamases were detected in 40 (39.6%), 33 (32.7%) and 29 (28.7%) isolates respectively. Eleven strains of *K. pneumoniae* and 1 strain of *Enterobacter cloacae* harbored all three genes.

Due to high immunosuppression, the active surveillance is absolutely crucial, because of infectious complications and antibiotic resistance, which is serious and increasing problem, especially among *Enterobacteriaceae*.

The study was supported by the "Research Support Foundation Vaduz - Comprehensive Study at the Issue of Oncological Diseases"

FEMS7-2432
Pathogens / Pathogenicity - Part II

HIGH SEROPREVALENCE OF TOXOPLASMA GONDII IN WILD RATS IN NORTH OF IRAN

S.A. Hosseini[1], A. Ahmad Daryani[2], A. Afsaneh Amouei[2], A. Azadeh Mizani[2], M. Mehdi Sharif[2]
[1]Student research Committee- Mazandaran University of Medical Sciences- Sari- Mazandaran- 48178 44718- Iran, Parasitology and Mycology, Sari, Iran
[2]Toxoplasmosis Research Center- Mazandaran University of Medical Sciences- Sari- Mazandaran- 4847191971- Iran, Parasitology and Mycology, Sari, Iran

Backgrounds

Toxoplasma gondii, an intracellular parasite, is one of the most common parasitic diseases of animals and man. Rats as herbivores are susceptible to *T*. *gondii* infection due to ingestion food or water contaminated with oocysts. The determination of the prevalence of *T*. *gondii* infection in rodents may be of epidemiological importance because rodents can serve as sources of tissue cysts for Felidae.

Objectives

The aim of the present investigation was to survey on seroprevalence of *T*. *gondii* in wild rats in Sari city, Mazandaran, north of Iran.

Methods

The seroprevalence of T. gondii determined among wild rats (R. rattus) in north of Iran from April to December 2014. Modified agglutination test (MAT) to detect serum antibodies against T. gondii was performed for 100 adult wild rats. The rats were classified according to sex and age and habit type. The results were analyzed by Chi-square analysis and Fisher's exact test.

Conclusions

Antibodies IgG anti- Toxoplasma gondii were detected in sera of 56 of 100 rats in titers of 1:40 in 2, 1:80 in 34, 1:160 in 14, 1:320 in 5 and 1:640 in 1 samples. Our findings confirms the high prevalence of toxoplasmosis in north of Iran. This study concluded that wild rats represent a significant and persistent wildlife intermediate host reservoir for T.gondii. Therefore, infected rats play an important role in the transmission of toxoplasmosis among animals and hence integrated strategies and measures should be taken to control toxoplasmosis in wild rats.

FEMS7-0715
Pathogens / Pathogenicity - Part II

CANDIDA ALBICANS SFP1 IN THE REGULATION OF BIOFILM FORMATION AND DRUG RESISTANCE

C. Hsueh-Fen[1], *C.Y. Lan*[2]
[1]*National Tsing Hua University, Hsinchu, Taiwan R.O.C.*
[2]*National Tsing Hua University, Institute of Molecular and Cellular Biology, Hsinchu, Taiwan R.O.C.*

Backgrounds

Candida albicans is a major human fungal pathogen. One of the important features of *C*. *albicans* pathogenicity is its ability to form biofilm. The cell surface of *C. albicans* adhere to supporting materials is critical for biofilm formation and device-associated infections.

Objectives

In this study, we studied the role of the transcription factors Sfp1 in biofilm formation and resistance to caspofungin, an antifungal agent targeting to cell wall.

Methods

The *SFP1*-deletion and *SFP1*-reintegrated mutant strains were generated. We compared wild type and the mutants in their ability in biofilm formation using XTT reduction assay, scanning electron microscopy and confocal laser scanning microscopy. We also examined the effects of *SFP1* deletion on cell wall properties and composition. Finally, cell susceptibility to caspofungin was determined using MIC and spot assay.

Conclusions

The *SFP1*-deletion mutants increased not only cell adhesion, biofilm formation but also caspofungin resistance. Our results provide new insights into the role of *C*. *albicans* Sfp1.

FEMS7-0525
Pathogens / Pathogenicity - Part II

A FASYN CLUSTER OUTSIDE THE LEE ISLAND AFFECTS EHEC BACTERIAL TYPE-III SECRETION THOUGH MULTIPLE REGULATORY SWITCHES

Y.H. Huang[1], W.J. Syu[1], S.T. Hu[1], I.T. Ling[1], H.Y. Tsai[1]
[1]National Yang-Ming University, Microbiology and Inmmunology, Taipei, Taiwan R.O.C.

Backgrounds

Comparing the two genomes between enterohaemorrhagic *Escherichia coli* (EHEC) and K-12 strain, we have previously identified a unique gene cluster named "*fasyn*" in EHEC and experimentally demonstrated that mutants with *fasyn*-deleted affects the type-3 secretion of EHEC. This *fasyn* cluster contains genes numbered Z4863, Z4864, Z4865, and Z4866.

Objectives

This study is to deduce the function(s) of *fasyn* and explore additional changes when *fasyn* is deleted.

Methods

Western blotting was used to compare the amounts of proteins synthesized and secreted with different strains. Bacterial two-hybrid system was used to examine whether two gene products are associated each other. Quantitative RT-PCR was applied to detect whether gene activators were regulated when *fasyn* is deleted at the transcriptional level. Minimal inhibitory concentration was measured to see whether gene deletion makes mutant change its sensitivities to antibiotics.

Conclusions

There was no strong pair-wise interaction among the four gene products encoded by the *fasyn* cluster as revealed by the bacterial two-hybrid assay. EHEC seemed to be more sensitive to rifampicin when *fasyn* was deleted. Furthermore, by qPCR, it was found that a down regulation happened to *ler*, the LEE island global activator, and to a few gene activators outside the LEE island such as *lrp*, *leuO*, and *kdpE*. Complementation with one *fasyn* gene at a time apparently could not restore well the original phenotypes. Therefore, EHEC with *fasyn* deleted may affect multiple gene regulatory switches so that unbalanced signals disturb the authentic bacterial phenotypes.

FEMS7-0191
Pathogens / Pathogenicity - Part II

SCREENING OF NOVEL BIOFILM- AND QUORUM SENSING-MODULATING COMPOUNDS

H.J. Hwang[1], *S.K. Kim*[1]
[1]*Pusan National University, Department of Pharmacy, Busan, Republic of Korea*

Backgrounds

Biofilms are bacterial group behaviors that provide cells with many biological advantages, such as high infectivity, antibiotic resistance, and strong survivability. Currently, most persistent bacterial infections are associated with antibiotic-resistant biofilms of pathogenic bacteria. Quorum sensing (QS) is a key regulation system that induces a large number of virulence genes. *Pseudomonas aeruginosa* is an opportunistic human pathogen whose biofilm formation and QS regulation cause great losses in many industrial facilities and serious infections in humans. Therefore, controls of the *P. aeruginosa* biofilm and QS response are a very important issue in medicine, public health, and industry.

Objectives

We synthesized and screened a series of novel compounds for anti-biofilm and anti-QS activities.

Methods

To screen the compounds, we used static biofilm assays and bioassays using several reporter strains. A *cdrA-lacZ* fusion reporter was used for the measurement of the intracellular c-di-GMP level, and *lasI-lacZ* and *PA1897-lacZ* fusions, for the measurement of the QS activity.

Conclusions

We found that several synthetic compounds such as MHY384, MHY1329, MHY1344, MHY1351, and MHY1354 had a significantly modulating activity on the biofilm formation and QS of *P. aeruginosa*.

FEMS7-2266
Pathogens / Pathogenicity - Part II

S. AUREUS ADAPTATION DURING PNEUMONIA IN MURINE MODEL: MODULATION OF EXPRESSION OF A SELECTED SET OF GENES

R. Iantomasi[1], D. Seguin[1], N. Arnaud Barbe[1], V. Sanchez[1], C. Chabanel[1], C. Gregoire[1], B. Rokbi[1]
[1]Sanofi Pasteur, R&D, Marcy-l'Étoile, France

Backgrounds

Staphylococcus aureus is a gram-positive opportunistic bacteria considered as the major pathogen in community-acquired and nosocomial infections and it has the potential to cause a broad range of disease. The pronounced adaptability to diverse physiologic niches resides in the vast array of virulent factors coded by *S. aureus* which needs to be deeply investigated in order to prevent specific infections.

Objectives

In this study, we investigated the in vivo transcriptional adaptation of *S. aureus* during pneumonia infection.

Methods

Female BalbC mice were infected via intranasal route with *S. aureus* USA 300 strain in order to mimic pneumonia infection. At 5h and 24h post infection (p.i.) infected lungs were collected and processed for prokaryotic RNA extraction. Gene expression was assessed by Fluidigm dynamic array on the BioMark™ HD System allowing quantitative high-throughput real-time PCR analysis. A set of 56 genes covering the mechanisms of replication/adhesion, biofilm formation, iron uptake/transport and immune evasion/stress response were included, as well as regulators factors and housekeeping genes.

Conclusions

The ability of *S. aureus* to adapt to extreme changes, like *in vitro* vs *in vivo*, implies the finely regulation of genes expression. In pneumonia model we observed the up-regulation of genes involved in the adhesion mechanisms, in particularly the MSCRAMM family, and iron acquisition since early time point post infection. *hla*, an important *S. aureus* α-toxin was also found expressed up to 24h post infection. The design and the development of a prevention strategy as well as infection treatment, must take into account the bacterial “physiological” state during infection.

FEMS7-0479
Pathogens / Pathogenicity - Part II

PRECISION MICROBIOTA RECONSTITUTION AFTER ANTIBIOTIC THERAPY CONFERS RESISTANCE AGAINST VANCOMYCIN-RESISTANT ENTEROCOCCUS

S. Isaac[1], A. Djucovik[1], L. Puchades-Carrasco[2], A. Flores-Duro[1], J. Pons[1], N. Jiménez[1], A. Pineda-Lucena[2,3], M. Garcia-Garcerà[4], C. Ubeda[1,5]
[1]Fundación para el Fomento de la Investigación Sanitaria y Biomédica FISABIO, Genomics and Health, Valencia, Spain
[2]Centro de Investigación Príncipe Felipe, Structural Biochemistry Laboratory, Valencia, Spain
[3]Hospital Universitario i Politécnico La Fe, Instituto de Investigación Sanitaria La Fe, Valencia, Spain
[4]Université de Lausanne, Département de microbiologie fondamentale, Lausanne, Switzerland
[5]Centers of Biomedical Research Network CIBER in Epidemiology and Public Health, CIBEResp, Madrid, Spain

Backgrounds

Commensal bacteria are able to inhibit intestinal colonization by opportunistic pathogens, such as vancomycin-resistant Enterococcus (VRE). Antibiotics alter the microbiota and promote gut colonization by VRE. However, the specific commensal bacteria that protect against VRE and the mechanisms of such protection have not been defined yet.

Objectives

Our objectives are to identify members of the intestinal microbiota that confer protection against VRE and the mechanisms of such protection.

Methods

We used a VRE infection mouse model and 16s rRNA high-throughput sequencing to identify commensal bacteria that are associated with resistance to VRE in mice. We isolated and administered these bacteria to vancomycin-treated mice to test their capability to restore colonization resistance.
We used metatranscriptomic and metabolomic approaches to identify in vivo functions expressed and metabolites produced by protective commensal bacteria that could be involved in protection against VRE intestinal colonization.

Conclusions

We demonstrated that the administration of 5 bacterial isolates to mice (*Alistipes, Barnesiella, Olsenella, Oscillibacter* and a bacterium from Ruminococcaceae family) restores protection to VRE in antibiotic-treated mice. The mechanism of such protection may involved nutritional competition for carbohydrates (i.e. mannose or cellobiose) since: (i) protective bacteria diminish the intestinal availability of carbohydrates in antibiotic-treated mice, (ii) expression of transporters for internalization of mannose/cellobiose increases in vancomycin-treated mice upon administration of the five protective strains, (iii) within carbohydrates transporters expressed by VRE in the murine gut, cellobiose and mannose transporters are among the most expressed, (iv) *in vitro* VRE grows efficiently using cellobiose or mannose as unique carbon sources.

FEMS7-1752
Pathogens / Pathogenicity - Part II

MYCOPHAGOUS LIFESTYLE OF BACILLUS SPHAERICUS AGAINST THE PHYTOPATHOGENIC FUNGUS RHIZOCTONIA SOLANI

I. Jamil[1], *S. Bindschedler*[1], *P. Junier*[1]
[1]*University of Neuchâtel, Laboratory of Microbiology, Neuchâtel, Switzerland*

Backgrounds

Bacteria are well known for their diversified nutritional capabilities. Many of these either arise from, or give origin to, interactions with other organisms. In some conditions, the nutritional ability of the bacterium can favor positive or negative interactions with other organisms. Mycophagy represents a series of phenotypic behaviors allowing bacteria to obtain nutrients from living fungi with a negative impact on the fungal partner. Soil is the main hub of bacterial-fungal interactions. Among bacterial soil dwellers, Bacilli are considered to be important components of the soil microbial community. This group includes species widely used in bio fertilization and biocontrol.

Objectives

To investigate the interaction between *Bacillus sphaericus* and the phytopathogenic fungus *Rizoctonia solani* in order to understand an unexplored mycophagous lifestyle and its potential use in sustainable agriculture.

Methods

In order to understand the mechanisms underlying this mycophagous lifestyle, a confrontation assay was performed, where *Rhizoctonia solani* biomass and its exudates were the sole carbon source for the bacteria.

Conclusions

Bacillus sphaericus inhibited fungal growth and used fungal hyphae for their dispersal. Fungal tissues that were in direct contact with bacteria were adversely damaged and unable to re-grow. Microscopic observations revealed both the deformation and the disintegration of the fungal cell wall. Moreover, bacterial growth occurred at the expense of fungal mycelium but no growth was measured in fungal exudates confirming that living mycelium is required for bacterial growth. This yet unexplored mycophagous lifestyle of *Bacillus sphaericus* could be exploited for the control of phytopathogens in sustainable agriculture.

FEMS7-1933
Pathogens / Pathogenicity - Part II

IDENTIFICATION OF GENETIC PATHWAYS FOR FORMATION OF INTRACELLULAR PERSISTERS OF SALMONELLA TYPHIMURIUM

K. Lauridsen[1], L. Jelsbak[1]
[1]Roskilde University, Science- Systems and Models, Roskilde, Denmark

Backgrounds

It is well-known that during growth in laboratory media (*in vitro*) bacteria are able to form a sub-population of non-growing bacteria (so-called persister cells), which, due to their inactive nature, exhibit a transient tolerance to treatment with antibiotics. The molecular mechanisms of *in vitro* persister cell formation have been studied intensively over the last decade. In recent studies, the presence of intracellular persisters of *Salmonella Typhimurium* bacteria infecting cultured macrophages has been reported (Helaine and co-workers).

Objectives

The aim of this study was to identify the genetic determinants of *in vivo* persisters of *S. Typhimurium* and investigate whether the host environment affects the mechanisms/pathways by which persister cells are formed.

Methods

In the present project we have screened a transposon library for mutants with increased persistence following infection of cultured macrophages (*in vivo*) and compared these results with a similar screen for increased persistence in Laboratory Growth media (*in vitro*). We have isolated ten mutants from each screen and are now further characterizing these with respect to persistence and growth physiology. We will determine the genetic determinants by whole genome sequencing of isolated mutants.

Conclusions

Based on our preliminary investigations, the two types of persister mutants (*in vitro* versus *in vivo*) exhibit distinct characteristics. Upon the results of the genome sequencing, we will further investigate the molecular mechanisms of both types of persister mutants, and these data will also be presented at the conference.

FEMS7-1116
Pathogens / Pathogenicity - Part II

THE B COMPONENT OF THE BACILLUS CEREUS HBL ENTEROTOXIN COMPLEX DETERMINES THE TOXIC ACTIVITY TOWARDS DIFFERENT TARGET CELLS

N. Jessberger[1], R. Dietrich[1], F. Tausch[1], A. Didier[1], E. Märtlbauer[1]
[1]Institute for Hygiene and Technology of Milk, Department of Veterinary Sciences, Oberschleissheim, Germany

Backgrounds

A major virulence factor for *Bacillus cereus* food poisoning is the three component enterotoxin haemolysin BL. It consists of the binding component B and the lytic components L2 and L1.

Objectives

Studying the mode of action of Hbl has been difficult, as natural culture supernatants additionally contain Nhe, the second three component enterotoxin.

Methods

In the present study, pore formation as well as cytotoxic and haemolytic activity of recombinant Hbl components was investigated.

Conclusions

rHbl was toxic to cell lines from different origin and tissue. All three components were necessary for cytotoxicity and pore formation. Excess of rHbl B enhanced, excess of rHbl L1 hindered velocity of pore formation. Pore formation was fastest at concentration ratios of L2:L1:B = 1:1:10 and 10:1:10, while a ration of L2:L1:B of 1:1:1 resulted in maximum toxic activity. Hbl activity was due to sequential binding of B – L1 – L2. Binding of Hbl B to the cell surface took 4-5 min, while apposition of L1 and L2 occurred immediately. Diluting the individual components, it became obvious that binding of the B component to the target cell surface is the crucial step for pore formation.

These data suggest that the mechanism of pore formation of Hbl differs from that of Nhe, although both enterotoxin complexes are sequentially highly related.

The IGF Project 18677 N of the FEI is supported via AiF within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament.

FEMS7-1371
Pathogens / Pathogenicity - Part II

EFFECTS OF THE BILE SALT SODIUM GLYCOCHOLATE HYDRATE ON GLOBAL GENE AND PROTEIN EXPRESSION IN CLINICAL ENTEROTOXIGENIC ESCHERICHIA COLI (ETEC) ISOLATES

E. Joffré[1], M. Nicklasson[2], S. Alvarez-Carretero[1], X. Xiao[3], B. Zhu[3], Å. Sjöling[1]
[1]Karolinska Institutet, Microbiology- Tumor and Cell Biology MTC, Stockholm, Sweden
[2]University of Gothenburg, Institute of Biomedicine- Department of Microbiology and Immunology, Göteborg, Sweden
[3]Chinese Academy of Sciences- Beijing,
CAS Key Laboratory of Pathogenic Microbiology & Immunology- Institute of Microbiology, Beijing, China

Backgrounds

Enterotoxigenic E. coli (ETEC) is one of the most common bacterial causes of acute watery diarrhea in the developing world, both among children and adults. When ETEC enter into the host and pass through the intestinal tract, the bacteria encounter an array of host factors to avoid bacterial colonization and spreading. Bile is one of the host factors conferring protection against bacteria; however, certain concentration has been demonstrated to regulate virulence in several pathogens. Still little is known about how the gene expression of ETEC is modulated by bile.

Objectives

The goal of the present study was to further explore the contribution of bile, and in particular NaGCH, to global gene expression in ETEC strains expressing CS5 and CS6.

Methods

To address this, we used bile salts and an individual bile salts, sodium glycocholate hydrate (NaGCH) enriched media to grow two ETEC CS5+CS6 strains E1777 and E2265 and subsequently we performed a transcriptome and proteome analysis using RNA-seq and iTRAQ-coupled LC-MS/MS to measure the global gene expression, transcriptome and proteome variation in presence of bile and NaGCH.

Conclusions

The resulting gene expression profiles showed that virulence genes: CS5 operon (csfA, csfC and csfD), csvR and cexE, were strongly upregulated by both bile and NaGCH. Bile upregulated genes involved in fatty acid oxidation while NaGCH upregulated nitrogen metabolism, biofilm formation and chemotaxis genes. fliC gene involved in bacterial motility was downregulated by NaGCH. As was expected, the proteomic data confirmed the significant upregulation of CS5 and CexE. Our data demonstrate that bile and especially individual bile salts NaGCH upregulate the virulence in ETEC and but also modulate differentially ETEC gene expression of different pathways and additional factors that are directly or indirectly involved in ETEC pathogenesis.

FEMS7-2564
Pathogens / Pathogenicity - Part II

ENDOCARDITIS DUE TO STREPTOCOCCUS SUIS IN FARMED MINKS IN ICELAND

G. Jonsdottir[1]
[1]*The Institute for Experimental Pathology- University of Iceland- Keldur, Bacteriology and Pathology, Reykjavík, Iceland*

Backgrounds

Streptococcus suis (S. suis) is an opportunistic bacterium usually associated with pigs. The bacterium is found in the tonsils of most pigs and capable of causing meningitis and septicemia. *S. suis* has also been isolated from other animals, such as minks, horses, dogs, cats, and birds. The bacterium can cause disease both in animals and humans.

Objectives

The aim of this study is to describe endocarditis in farmed minks mainly caused by *S. suis.*

Methods

In the years 2010 to 2015 the Institute of Experimental Pathology, Keldur received 31 minks from one farm for post mortem examination.The minks had all been fed offal from a pig slaughterhouse and the feed had probably not been adequately heat-treated. Autopsy revealed vegetative endocarditis and *S. suis* was isolated in the majority of cases. Both *S. suis* serotype I and II were isolated.

Conclusions

Our study showed that death in minks due to endocarditis was primarily due to *S. suis*, either serotype I or II. Most of the minks acquired the infected in July to September and were found dead without clinical signs being observed. It is important to treat offal with caution especially if it's going to be used as animal feed. *S. suis* is a zoonotic agent that can be a serious pathogen both in animals and humans

FEMS7-1439
Pathogens / Pathogenicity - Part II

CHEMICAL QUORUM QUENCHING ATTENUATES RALSTONIA SOLANACEARUM VIRULENCE ON PLANTS

K. Kai[1], A. Yoshihara[1], M. Shimatani[1], Y. Hikichi[2]
[1]Osaka Prefecture University, Graduate School of Life and Environmental Sciences, Osaka, Japan
[2]Kochi University, Department of Agriculture, Kochi, Japan

Backgrounds

Ralstonia solanacearum is a beta-Proteobacterium bacterium that causes “bacterial wilt” on a wide range of plant species. Quorum sensing (QS), a bacterial cell-cell communication mechanism, controls virulence determinants, such as the production of extracellular polysaccharide (EPS) and biofilm formation, in *R. solanacearum*. The bacterial strains employ methyl 3-hydroxymyristate or methyl 3-hydroxypalmitate as the QS signals, which are synthesized and sensed by *phc* QS system.

Objectives

Here we describe the design, synthesis, and biological evaluation of the inhibitors of *phc* QS.

Methods

Initial screening of a small set of QS signal analogs found that PQI-1 (*phc* quorum sensing inhibitor-1), inhibits biofilm formation by *R. solanacearum*. To improve its inhibitory activity, the derivatives of PQI-1 were synthesized. PQI-2−5 inhibited not only biofilm formation but also other QS-controlled traits. Those antagonists reduced wilting symptoms of the tomato plants infected with *R. solanacearum.*

Conclusions

Targeting *phc* QS has potential for the development of agrochemicals that protect crops from bacterial wilt disease.

FEMS7-2187
Pathogens / Pathogenicity - Part II

IDENTIFICATION, FUNCTIONAL CHARACTERIZATION AND REGULON PREDICTION OF A NOVEL TWO COMPONENT SYSTEM COMPRISING BAS0540-BAS0541 OF BACILLUS ANTHRACIS

D. Kandari[1], *M. Gopalani*[1], *R. Bhatnagar*[1]
[1]*Jawaharlal Nehru University, School of Biotechnology, New Delhi, India*

Backgrounds

Two component systems (TCSs) comprise of a membrane bound sensor Histidine Kinase (HK), and a cytosolic Response Regulator (RR). TCSs are complex molecular devices that help bacteria sense its environment and respond aptly. 41 TCSs are predicted in *Bacillus anthracis*, a potential bioterrorism agent, of which only four have been studied so far.

Objectives

Implication of TCSs in the maintenance of a specialized lifestyle pattern and pathogenesis of B. anthracis remains largely unmapped and needs comprehensive exploration. Our study characterizes a novel TCS of B. anthracis and elucidates its role in the physiology of the pathogen.

Methods

qRT-PCR, immunoblotting, autophosphorylation/phosphotransfer assays, EMSA, electroporation, confocal/DIC microscopy.

Conclusions

BAS0540-BAS0541 constitutes a classical TCS of *B. anthracis*. The RR and HK are encoded by BAS0540 and BAS0541, respectively. BAS0541 exhibited autophosphorylation and phosphotransfer to its cognate RR BAS0540. BAS0540 exhibited a constitutive expression throughout the growth of *B. anthracis*. The *in silico* predicted regulon of BAS0540 comprised of 23 genes with a diverse set of functions excluding any of the proven virulence factors. EMSA demonstrated the direct binding of BAS0540 to the upstream regions of functionally important putative regulon genes like *ftsA*. Phosphorylation by small molecule phosphodonors led to a striking enhancement in the DNA binding capability of BAS0540. Overexpression of BAS0540 in *B. anthracis* led to a prodigious increase in the cell length and a discernible decrease in the sporulation efficiency of the bacteria. Thus, BAS0540-BAS0541 forms a functional TCS of *B. anthracis* which is implicated in cell division and sporulation of the pathogen.

FEMS7-1125
Pathogens / Pathogenicity - Part II

ANTIMICROBIAL AND ANTIBIOFILM ACTIVITIES OF MODIFIED-ANTIMICROBIAL PEPTIDE AGAINST BURKHOLDERIA PSEUDOMALLEI

S. Kanthawong[1], S. Wongkaewkhiaw[2], J.G.M. Bolscher[3], K. Nazmi[3], S. Wongratanacheewin[2], C. Anutrakunchai[4], S. Taweechaisupapong[5]
[1]Biofilm Research Group and Faculty of Medicine/Khon Kaen University, Department of Microbiology, Khon Kaen, Thailand
[2]Melioidosis Research Center MRC- Faculty of Medicine/Khon Kaen University, Department of Microbiology, Khon Kaen, Thailand
[3]Academic Centre for Dentistry Amsterdam ACTA, Department of Oral Biochemistry, Amsterdam, The Netherlands
[4]Biofilm Research Group and Faculty of Medicine/Khon Kaen University, Department of Biochemistry, Khon Kaen, Thailand
[5]Biofilm Research Group- Faculty of Dentistry/Khon Kaen University, Department of Oral Diagnosis, Khon Kaen, Thailand

Backgrounds

Melioidosis is a severe disease caused by *Burkholderia pseudomallei*. The growing *B. pseudomallei* in biofilm has been reported to induce resistance to several conventional antibiotics, a phenomenon that may be related to relapse cases in melioidosis patients.

Objectives

The aim of this study was to compare the antibiofilm activity of human cathelicidin antimicrobial peptides (LL-37, LL-31) and theirs D-enantiomeric form (D-LL-37, D-LL-31) against 3 isolates of *B. pseudomallei* 1026b, H777 and biofilm mutant M10.

Methods

Antibiofilm assay revealed that, a truncated variant of LL-37 lacking the six C-terminus residues in D-enatiomeric form, D-LL-31 revealed a strongest killing activity against all isolates of *B. pseudomallei* in dose-dependent manner. Moreover, the IC_{50} values of D-LL-31 was further investigated compared with CAZ against biofilm form of those isolates. The results showed that IC_{50} values of D-LL-31 were ranging from 1.07-5.55 µM, while, IC_{50} values of CAZ was higher than D-LL-31 about 45, 60 and 240 folds against biofilm form *B. pseudomallei* M10, H777 and 1026b, respectively. In addition, D-LL-31 showed strongly effects on biofilm of all isolates tested in static condition. Moreover, D-LL-31 was applied to biofilm of *B. pseudomallei* under BioFlux flowthrough conditions and caused disruption of the biofilms.

Conclusions

These results indicate that D-LL-31 not only disrupted preformed-biofilm but also exhibited potent killing activity against *B. pseudomallei* in biofilm form. Thus D-LL-31 should be considered to develop as novel antibiofilm agent against *B. pseudomallei* which is finally benefited to melioidosis patients.

FEMS7-3262
Pathogens / Pathogenicity - Part II

KINETIC AND THERMODYNAMIC STUDIES ON INTERACTIONS BETWEEN HUMAN PLASMINOGEN AND CANDIDA TROPICALIS CELL WALL-ASSOCIATED ENOLASE AND HYPHALLY REGULATED PROTEIN

J. Karkowska-Kuleta[1], D. Zajac[2], G. Bras[1], O. Bochenska[2], A. Kozik[2], M. Rapala-Kozik[1]
[1]Jagiellonian University - Faculty of Biochemistry- Biophysics and Biotechnology, Department of Comparative Biochemistry and Bioanalytics, Krakow, Poland
[2]Jagiellonian University - Faculty of Biochemistry- Biophysics and Biotechnology, Department of Analytical Biochemistry, Krakow, Poland

Backgrounds

Recruitment of human proteolytic enzymes at the cell surface of pathogenic microorganisms gives them an incredible opportunity to acquire additional hydrolytic activity. Hence, binding of human plasminogen (HPG) by the surface-exposed proteins might be an important mechanism increasing also the virulence of *Candida tropicalis*, now regarded as a third, or even second *Candida* spp. most commonly isolated from the bloodstream of immunocompromised individuals suffering from candidemia.

Objectives

The detailed characterization of interactions between HPG and *C. tropicalis* surface-exposed proteins.

Methods

To select fungal HGP-binding proteins, affinity chromatography was performed with the use of the mixture of *C. tropicalis* beta-1,6-glucanase-extractable cell wall proteins. Then, fungal enolase (Eno) and hyphally regulated protein (Hyr) were purified with the ion exchange chromatography. The kinetic and thermodynamic characterization of interactions between human plasminogen and each fungal protein was performed with the use of BIACORE 3000 system.

Conclusions

The dissociation constants determined for complexes of HPG and Hyr, a typical, highly glycosylated GPI-anchored hyphal cell wall protein, or Eno, a glycolytic enzyme that moonlight at the cell surface, were of a 10^{-7} M order, indicating rather a moderate binding affinity. Since surface-bound plasmin might greatly facilitate fungal invasion on human organism, the detailed characterization of interactions between this human protein and *C. tropicalis* surface-exposed proteins can significantly increase the understanding of the molecular bases of pathogenesis of infections caused by these fungi.

This work was supported by the Jagiellonian University in Krakow (Statutory Funds of the Faculty of Biochemistry, Biophysics and Biotechnology BMN29/2016 awarded to JKK).

FEMS7-0394
Pathogens / Pathogenicity - Part II

IMPORTANCE OF FUMARATE AND NITRATE REDUCTION REGULATORY PROTEIN FOR INTESTINAL PROLIFERATION OF VIBRIO VULNIFICUS IN INTESTINE

T. Kado[1], T. KASHIMOTO[1], K. Yamazaki[1], S. Ueno[1]
[1]Kitasato Univ. School of Veterinary Medicine, Veterinary Public Health, Towada, Japan

Backgrounds

The sepsis caused by *Vibrio vulnificus* is characterized by an average incubation period of 26 hours and a high mortality rate exceeding 50 %. The fast growth and dissemination of *V. vulnificus in vivo* lead to poor clinical outcomes in patients. Therefore, elucidation of the proliferation mechanisms of this organism *in vivo* may lead to development of an effective therapeutic strategy.

Objectives

In this study, we focused on the low oxygen concentration in the intestinal milieu because of its drastic difference from that in the air. Fumarate and nitrate reduction regulatory protein (FNR) has been known as the global transcriptional regulator for adaptation to anaerobic conditions in various bacteria.

Methods

We generated a strain of *V. vulnificus* in which the *fnr* gene was replaced with an erythromycin resistance gene (*fnr*::*erm* mutant). And the *fnr*::*erm* mutant was tested in a growth competition assay against WT *in vivo.*

Conclusions

The competitive index of *fnr*::*erm* mutant to WT in the intestinal loop and liver was 0.378±0.192 (Mean ± S.D) and 0.243±0.123 respectively. These data suggested that FNR is important for the proliferation of *V. vulnificus* in the intestine to achieve a critical mass to be able to invade systemic circulation.

FEMS7-0811
Pathogens / Pathogenicity - Part II

STOCHASTIC EXPRESSION OF LACTATE DEHYDROGENASE CAUSES E. COLI PERSISTER FORMATION

Y. Kawai[1], *R. Isshiki*[1], *N. Yamamoto*[1], *S. Matsumoto*[2], *S. Tsuneda*[1]
[1]*Waseda University, Department of Life Science and Medical Bioscience, Tokyo, Japan*
[2]*Nagoya University, Medicine, Nagoya, Japan*

Backgrounds

Persisters are multidrug tolerant cells that present within antibiotic sensitive population. In contrast to antibiotic resistant bacteria, the tolerance arises from transient phenotypic variants rather than genetic mutations. Cells which express persister gene stochastically can switch to persister states. Although previous study identified many genes involved in persister formation, molecular mechanisms of bacterial persistence remain unclear because of their redundancy. In order to identify novel persister genes, we developed a marker for *E.coli* persisters and performed transcriptome analysis of isolated persisters.

Objectives

The objective is to reveal molecular mechanisms of persister formation via lactate dehydrogenase (*ldhA*) which was identified from our transcriptome data.

Methods

Transcriptome data suggested expression of anaerobic respiration genes. We screened overexpression and knockdown strains derived from *E.coli* MG1655. CRISPR interference was used for gene knockdown. To visualize *ldhA* expression, YFP was cloned into pSC101 vector that contained *ldhA* promoter. The YFP fluorescence of reporter strain was analyzed by microfluidic devise.

Conclusions

ldhA overexpression increased persister population by 1000 times, and knockdown decrease the population by 10 times. Stochastic expression of *ldhA* may influence bacterial metabolic activity because *ldhA* uses central metabolite pyruvate and NADH. Time-lapse microscopy images of *ldhA* reporter strain showed the most of cells did not express *ldhA*, but a small portion of cells (~1%) highly expressed *ldhA*. These cells stopped dividing and tolerated lethal concentration of ampicillin. Interestingly, although *ldhA* was expressed transiently, the cells showed dormant phenotype over 1 hour. These results suggest that stochastic expression of *ldhA* triggers persister formation.

FEMS7-2946
Pathogens / Pathogenicity - Part II

XTT ASSAY FOR EVALUATING THE EFFECT OF BASSIATIN AS A NOVEL ANTISEPTIC AGENT ON HOSPITAL-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) AND STREPTOCOCCUS PNEUMONIAE

E. Ozgor[1], N. Keskin[1], I. Celebier[1], M. Ulusoy[1]
[1]Hacettepe University Faculty Of Science, Biology, Ankara, Turkey

Backgrounds

Methicillin-resistant Staphylococcus aureus (MRSA) and *Streptococcus pneumoniae* are responsible for several serious infections in humans. Resistance does make infections more difficult to treat with standard types of antibiotics and thus more dangerous. Bassiatin is a newly discovered depsipeptide which is produced by fungal species. Bassiatin was observed that it inhibits cell proliferation and cell cycle progression in some cancer cell lines. Besides these studies, there is no another research with bassiatin.

Objectives

Objective of this study is investigate the effects of bassiatin for microbial inhibition on clinical MRSA and *S.pneumoniae* isolates from private hospital in Turkey.

Methods

Bassiatin which is produced by *Beauveria bassiana* was purified. *B.bassiana* was grown in specific medium at pH 6.5 under 28°. The extract of the culture broth afforded 150 mg of residue under reduced pressure and it was further separated by HPLC to give bassiatin. The XTT colorimetric assay was used to determine the percentage of viable cells following each treatment. For bacterial culture preparation, 200 µl of overnight culture adjusted to 5×10^5 cells/ml was added to 96-well plate. Following 17 h treatment with different concentrations of bassiatin, 100 µl from each well was transferred to new plate and 25 µl XTT/Menadione was added on them. After an incubation period of 1h at 37°C, the absorbance at 490 nm was measured.

Conclusions

The antimicrobial activity of bassiatin was evaluated in accordance to a predefined threshold of a minimum of 50% reduction in the produced formazan with XTT colorimetry assay. Bassiatin was found to be effective on MRSA and *S.pneumoniae*. Furthermore, it was found that there is a correlation between the concentration of Bassiatin and inhibition of MRSA and *S.pneumoniae*. Consequently, this study is promising for further clinical studies and bassiatin may be used as a potential drug against MRSA and *S.pneumoniae* in future.

FEMS7-0238
Pathogens / Pathogenicity - Part II

CYCLO-(L-PHE-L-PRO) INDUCES EXPRESSION OF HYDROPEROXIDASE BY REGULATION OF RPOS TO FACILITATE THE SURVIVIAL OF PATHOGENIC VIBRIO SPP UNDER OXIDATIVE STRESS

K.S. Kim[1]
[1]*Sogang University, Department of Life Science, Seoul, Republic of Korea*

Backgrounds

Vibrio vulnificus, an opportunistic human pathogen, produces cyclo (L-Phe-L-Pro) (cFP), which serves as a signal molecule controlling the ToxR-dependent expression of innate bacterial genes, and as a virulence factor eliciting pathogenic symptom on human cells by enhancing the intracellular reactive oxygen species level. We found that cFP facilitates the survival of *V. vulnificus* under the oxidative stress induced by H_2O_2.

Objectives

Molecular mechanisms underlying the cFP-mediated resistance against the oxidative stress induced by H_2O_2 were to be elucidated.

Methods

Employing transcriptomic analyses and also various molecular genetic tools, we studied regulatory components associated with cFP-mediated resistance against oxidative stress.

Conclusions

We found a novel cFP-LeuO-vHUαβ-RpoS pathway that is responsible for the induction of *katG* encoding hydroperoxidase I that detoxifies H_2O_2 to overcome the oxidative stress condition. This pathway is also up-regulates genes known to be members of the RpoS regulon, suggesting that cFP is a cue for a signal transduction pathway responsible for regulons of LeuO as well as RpoS.

FEMS7-1988
Pathogens / Pathogenicity - Part II

GENETIC RELATIONSHIPS OF ERWINIA AMYLOVORA ISOLATES OCCURRED IN KOREA

S.H. Kim[1], B.Y. Kim[1], Y.H. Yun[1], J.Y. Kim[1], S.J. Lee[2], O.K. Jung[3]
[1]Dankook University, Microbiology Department, Cheonan, Republic of Korea
[2]Animal and Plant Quarantine Agency, Plant Pest Control Department, Gimcheon, Republic of Korea
[3]Cheonan-si Agricultural Technology Center, Fruit Tree Department, Cheonan, Republic of Korea

Backgrounds

Erwinia amylovora, a Gram-negative bacterium in the family enterobacteriaceae, causes fire blight disease in apples and pears. In 2015, fire blight disease occurred for the first time in pear and apple orchards in Korea.

Objectives

A total of 64 *Erwinia amylovora* isolates were obtained from 40 farms located in Anseong, Cheonan, and Jecheon regions and their genetic relationships were studied with foreign isolates to understand their potent origin.

Methods

All the Korean isolates analyzed showed identical nucleotide sequence in the 16S rRNA, *gyr*B (DNA gyrase subunit B), and *rpo*D (RNA polymerase sigma factor RpoD) genes. When the pattern of clustered regularly interspaced short palindromic repeats (CRISPR) which have three spacer regions in *E. amylovora* was compared between the Korean isolates and 122 isolates from 14 countries, all the Korean isolates were analyzed as the 2-22-38 genotype that includes four isolates from USA and Canada. When the nucleotide sequence of six loci in variable number of tandem repeats (VNTR) was analyzed, five genotypes were resolved in the 64 Korean *E. amylovora* isolates. Among the five genotypes, the genotype 1 was found in 73% of the isolates.

Conclusions

When VNTR analysis of the Korean isolates was extended with foreign *E. amylovora* isolates from 26 countries, Korean isolates of the genotype 2 agreed with one isolate from Canada which belongs to the CRISPR genotype 2-22-38, revealing that the results of VNTR analysis supports those of CRISPR analysis. Overall, Korean *E. amylovora* isolates are genetically close to North American *E. amylovora* isolates.

FEMS7-3107
Pathogens / Pathogenicity - Part II

GROWTH PROPERTIES OF KOREAN ISOLATES OF FIRE BLIGHT PATHOGEN

S.H. Kim[1], B.Y. Kim[1], Y.H. Yun[1], O.K. Jung[2]
[1]Dankook University, Microbiology Department, Cheonan, Republic of Korea
[2]Cheonan-si Agricultural Technology Center, Pomology, Cheonan, Republic of Korea

Backgrounds

In 2015, fire blight disease occurred in pear and apple trees in Korea. From the investigation of the disease occurred farms in Anseong, Cheonan, and Jecheon regions, 64 *Erwinia amylovora* were obtained and they were divided into five genotypes by the analysis of the pattern of variable number of tandem repeats (VNTR).

Objectives

The present study was performed to investigate the growth properties of these five types of Korean *E. amylovora* isolates.

Methods

Five isolates which were randomly selected from each VNTR genotype were used for the investigation. Their ability of utilizing 20 different nutrient substrates were tested using an API kit. For the production of extracellular enzymes, they were grown on chromagenic media supplemented with high molecular substrates such as pectin, amylose, xylan, CM-cellulose, avicel, cellobiose, and skim milk. Sensitivity to antibiotics was assessed by the disc-diffusion assay.

Conclusions

They all could utilize only six substrates including D-glucose, D-mannitol, N-acetyl-glucosamine, malic acid, and potassium gluconate. They could not reduce NO3 but utilize L-tryptophan. There was no apparent difference among the five genotype isolates. They grew well at 25, 30, and 37 ℃. All the five *E. amylovora* isolates could not grown on chromogenic media supplemented with high molecular substrates, suggesting they have no ability of producing extracellular enzymes for these substrates. All the five types of *E. amylovora* isolates showed sensitivity to streptomycin and no difference was found in the degree of sensitivity to the antibiotics among them.

FEMS7-0159
Pathogens / Pathogenicity - Part II

CHANGES IN MEMBRANE CHARACTERISTICS WITH INCREASE OF ORNITHINE LIPID MODIFY THE INFECTIVITY OF P. AERUGINOSA

S.K. Kim[1], X.H. Li[1], J.H. Lee[1]
[1]Pusan National University, Department Of Pharmacy, Busan, Republic Of Korea

Backgrounds

Ornithine lipids (OLs) are bacteria-specific lipids that are widely found in outer membrane of many Gram (-) bacteria, but not detected in Eukarya and Archaea. Some bacteria produce OL to change the membrane composition in certain unusual situations like phosphate-limiting condition. *Pseudomonas aeruginosa* has *olsBA* operon for the OL biosynthesis to meet the phosphate limitation.

Objectives

We addressed how the OL production and resulting change of the lipid composition modulate the virulence of *P. aeruginosa* during the infection to host cells.

Methods

We controlled the OL level by overexpressing *olsBA* and confirmed the elevated level of OL by TLC and HPLC analyses. When the virulence of *P. aeruginosa* was investigated using two host models, *Tenebrio molitor* (insect) and *Caenorhabditis elegans* (nematode), the increase of OLs alleviated the virulence of *P. aeruginosa.* The host response to OLs was monitored through the expressions of COX-2 and iNOS using Western blot and real-time PCR analyses.

Conclusions

The elevated level of OL in membrane of *P. aeruginosa* increased hydrophobicity and positive charge of cell surface, which resulted in significant change of susceptibility of *P. aeruginosa* cells to antibiotics and host immunity, such as antimicrobial peptides and macrophages. OLs reduced the production of inflammatory factors such as iNOS, COX-2, PGE_2, and nitric oxide in host cells. Also, OL can increase the resistance to antimicrobial peptides such as LL-37 and magainin. The increase of OL content in *P. aeruginosa* modifies virulence by changing the cell surface property.

FEMS7-0812
Pathogens / Pathogenicity - Part II

INTERACTION BETWEEN THE TYPE III EFFECTOR VOPO AND GEF-H1 ACTIVATES THE RHOA-ROCK PATHWAY

H. Hiyoshi[1], S. Matsuda[2], K. Gotoh[3], T. Iida[2], T. Kodama[2]
[1]School of Medicine- University of California at Davis, Medical Microbiology and Immunology, Davis, USA
[2]RIMD - Osaka University, Bacterial Infections, Osaka-Suita, Japan
[3]Okayama University Graduate School of Medicine, Bacteriology, Okayama, Japan

Backgrounds

Vibrio parahaemolyticus is an important pathogen that causes food-borne gastroenteritis in humans. The type III secretion system encoded on chromosome 2 (T3SS2) plays a critical role in the enterotoxic activity of this bacterium. Previous studies have demonstrated that T3SS2 induces actin stress fibers in various epithelial cell lines during infection. This stress fiber formation is strongly related to pathogenicity, but the mechanisms that underlie T3SS2-dependent actin stress fiber formation and the main effector have not been elucidated.

Objectives

Objective of this study is the identification and the functional characterization of T3SS2 effector responsible for stress fiber formation.

Methods

V. parahaemolyticus strain RIMD2210633 (KP-positive, serotype O3:K6) was used as a parent strain. A four-primer polymerase chain reaction (PCR) technique was used to engineer an in-frame deletion mutation.

Conclusions

We identified VopO as a critical T3SS2 effector protein that activates the RhoA-ROCK pathway, which is essential pathway for the induction of the T3SS2-dependent stress fiber formation. We also determined that GEF-H1, a RhoA guanine nucleotide exchange factor (GEF), directly binds VopO and is necessary for T3SS2-dependent stress fiber formation. The GEF-H1-binding activity of VopO via an alpha helix region correlated well with its stress fiber-inducing capacity. Furthermore, we showed that VopO is involved in the T3SS2-dependent disruption of the epithelial barrier. Thus, VopO hijacks the RhoA-ROCK pathway in a different manner compared with previously reported bacterial toxins and effectors that modulate the Rho GTPase signaling pathway.

FEMS7-1382
Pathogens / Pathogenicity - Part II

FARNESOL POTENTIATES TMPYP-MEDIATED PHOTODYNAMIC INACTIVATION OF STAPHYLOCOCCUS AUREUS

M. Kossakowska-Zwierucho[1], *J. Nakonieczna*[1]
[1]*Intercollegiate Faculty of Biotechnology- University of Gdansk and Medical University of Gdansk, Department of Biotechnology- Laboratory of Molecular Diagnostics, Gdansk, Poland*

Backgrounds

Photodynamic inactivation (PDI) utilizes light-activated photosensitizing compounds, which in the presence of oxygen trigger local generation of reactive oxygen species and lead to death of target cells. This method can be used to eradicate human pathogens independently on their antibiotic resistance pattern. However, in the case of *Staphylococcus aureus*, a strain-specific sensitivity to PDI is observed which hampers its practical application. To overcome this heterogeneity, PDI can be combined with adjuvants sensitizing bacteria to photoinactivation.

Objectives

To analyse a combined action of red light-activated cationic porphyrin TMPyP (5.10.15.20-tetrakis(1-methyl-4-pyridinio)porphyrin) and *trans-trans*-farnesol – a natural compound of plant origin. The research hypothesis is that farnesol acts as an adjuvant-like agent specifically sensitizing *S. aureus* to the action of light-activated TMPyP via yet unknown molecular mechanism.

Methods

To identify molecular elements triggered by synergistic action of red light-activated TMPyP and farnesol in *S. aureus,* the primary changes in gene expression are analyzed using Tn*917-lacZ* reporter system. This method is based on a random transposon mutagenesis in strain RN4220, coupled with insertion of promoterless β-galactosidase gene in order to identify up-regulated genes under sub-lethal PDI conditions.

Conclusions

Observed potentiation of PDI with farnesol appears as a novel strategy to combat multiresistant *S. aureus* infections. Obtained results constitute the first step in a characterization of the mechanism of PDI and farnesol combined antibacterial action.

FEMS7-1223
Pathogens / Pathogenicity - Part II

PSEUDOMONAS AERUGINOSA PHOSPHOLIPASES AND LECTINS ARE POTENTIAL THERAPEUTIC TARGETS

F. Kovacic[1], F. Bleffert[1], T. Schwabroch[1], C.H. Strunk[1], M. Caliskan[1], J. Granzin[2], M. Siebers[3], P. Dörmann[3], L. Rahme[4], R. Batra-Safferling[2], K.E. Jaeger[1,5]
[1]Institute of Molecular Enzyme Technology- Heinrich-Heine-University Düsseldorf, Bacterial Enzymology, Jülich, Germany
[2]Institute of Complex Systems- Forschungszentrum Jülich GmbH, ICS-6: Structural Biochemistry, Jülich, Germany
[3]Institute of Molecular Biotechnology- Universität Bonn, IMBIO, Bonn, Germany
[4]Harvard Medical School, Department of Microbiology and Immunology, Boston, USA
[5]Institute of Bio- and Geosciences- Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany

Backgrounds

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen infecting immunocompromised humans with high mortality rates. Many virulence factors, among them phospholipases and lectins contribute to biofilm formation and high antibiotic resistance of *P. aeruginosa*.

Objectives

Phospholipases are involved in bacterial adaptation, host membrane damage and modulation of host lipid signaling. We have studied a novel phospholipase A which may contribute to the biosynthesis of lipid messengers related to virulence and biofilm formation. The lectin LecB is located at the cell surface and mediates bacterial attachment to human tissue during initial biofilm formation. We have tried to elucidate the so far unknown LecB secretion pathway.

Methods

PlbF as a novel phospholipase A of *P. aeruginosa* was shown to hydrolyze membrane phospholipids *in vitro* and *in vivo* releasing medium chain fatty acids. It acts as a virulence factor as shown by a *Drosophila melanogaster* infection model and is involved in biofilm formation. Screening of a transposon mutant library of *P. aeruginosa* using a high-throughput enzyme-linked lectin assay resulted in the identification of approximately 30 strains significantly affected in LecB secretion with targeted genes involved in the biogenesis of the flagellum and type-IV pili involved in cell attachment and biofilm formation.

Conclusions

The novel phospholipase PlbF may be linked to a virulence mechanism involving cell signaling in *P. aeruginosa*. The lectin LecB may be co-secreted with flagellin *via* a type-III secretion system. These data suggest that that lectin and phospholipase may represent potential targets for treatment of *P. aeruginosa* infections.

FEMS7-3151
Pathogens / Pathogenicity - Part II

SELECTION OF AN ANTI-UBIQUITIN SSDNA APTAMER – A TOOL FOR STUDYING THE RELATIONSHIP BETWEEN UBIQUITINATION AND MICROBIAL INFECTIONS

E. Kowalska[1], K. Muszynska[1], F. Bartnicki[1], M. Bodaszewska-Lubas[1], W. Strzalka[1]
[1]Jagiellonian University, Plant Biotechnology, Krakow, Poland

Backgrounds

Ubiquitin is a well characterized conservative eukaryotic post-translational modifier (1). It was shown to be involved in signal transduction and to play a regulatory function in cellular processes. What is more ubiquitination turned out to be a key process in infectious disease responses in plants (2).

Objectives

To study the function of diverse ubiquitination patterns in plants, tools for detection and purification of ubiquitinated proteins are necessary. Therefore, the aim of this study was to develop a DNA aptamer recognizing ubiquitinated plant proteins.

Methods

GST-ubiquitin was overexpressed and purified to homogeneity from *E. coli* BL21 cells. The selection of a DNA aptamer was performed using the SELEX (*Systematic Evolution of Ligands by Exponential Enrichment*) method (3). The progress of the selection process was monitored using qPCR. After ten rounds of SELEX ssDNA molecules were cloned and sequenced. Among the selected ssDNA molecules five aptamer groups characterized by unique sequences were identified. The specificity and affinity of the identified aptamers were verified using qPCR and pull-down assays.

Conclusions

The developed aptamer may serve as a new tool for the study and analysis of the role of ubiquitination during plant microbial infections. This is crucial to better understand the molecular mechanisms of infectious diseases, since the modulation of host ubiquitination is a common mechanism of plant pathogen action.

Acknowledgments:

This work was financially supported by Jagiellonian University grant K/DSC/003699 to EK.

References

1. Callis,J. (2014) *Arabidopsis Book*, **12**, e0174.

2. Spallek et al. (2009) *Cellular Microbiology*, **11**, 1425–1434.

3. Tuerk,C. and Gold,L. (1990) *Science*, **249**, 505–510.

FEMS7-1567
Pathogens / Pathogenicity - Part II

SILVER NANOPARTICLES - NANOSIZED ENHANCERS OF THE ANTI-STAPHYLOCOCCAL ACTIVITY OF NAPHTHOQUINONES.

M. Krychowiak[1], *A. Kawiak*[1], *A. Królicka*[1]
[1]*Intercollegiate Faculty of Biotechnology UG&MUG, Department of Biotechnology, Gdansk, Poland*

Backgrounds

Staphylococcus aureus is a human pathogenic bacterium and a major cause of antibiotic-resistant infections. The failure of antibiotic-resistant therapies has driven the development of alternative approaches for combating microbial infections. Silver nanoparticles (AgNPs) and naturally occurring substances like naphthoquinones (NQs) represent antimicrobials with bactericidal potential towards *S. aureus*.

Objectives

The aim was to verify if silver nanoparticles enhance the anti-staphylococcal activity and decrease the cytotoxicity of selected naphthoquinones.

Methods

Antimicrobial potential of tested substances was determined by Broth Microdilutions Method (planktonic cultures) and MBEC Assay (bacterial biofilm). The Checkerboard Titration technique was employed to verify the impact of AgNPs on bactericidal potential of NQs. Cytotoxic effect of antimicrobials and their combinations was determined towards HaCaT cells with the use of the MTT Assay.

Conclusions

The overall results obtained during this study depicted the potential of AgNPs to enhance the bactericidal activity of NQs. The addition of AgNPs to culture medium lead to the reduction of NQs minimal bactericidal concentrations from 50 to 97%. Moreover, the decrease of NQs effective doses lead to a significant reduction of their cytotoxicity. What is most important, the enhancing effect of AgNPs was observed both for *S. aureus* strains resistant to antibiotics and staphylococcal biofilm.

FEMS7-3232
Pathogens / Pathogenicity - Part II

SEX IN AN ASEXUAL YEAST: GENOMIC EVIDENCE OF A SEXUAL CYCLE AND ERROR-PRONE MATING TYPE SWITCHING IN THE OPPORTUNISTIC PATHOGEN CANDIDA GLABRATA

E. Ksiezopolska[1], L. Carreté[1], C. Pegueroles[1], C. Fairhead[2], T. Gabaldon[1]
[1]Centre for Genomic Regulation CRG, Bioinformatics and Genomics, Barcelona, Spain
[2]Paris-Sud University, Institute for the Diversity- Ecology and Evolution of the Living World, Paris, France

Backgrounds

Candida glabrata is considered an asexual species despite maintaing homolog genes to those involved in mating in *Saccharomyces cerevisiae*. Debates about its sexuality have arisen as some research indicates that *C. glabrata*, similarly to *Candida albicans*, might be able to mate (Muller *et al.,* 2008), other claims that the MLST-based clades show a strong bias in the presence of only one of the two mating types, suggesting a low incidence of mating type switching (Brisse *et al.,* 2009).

Objectives

We aim to address whether *C. glabrata* is able to mate using whole genome data and to perform for the first time a genome-wide characterization of the population structure in this species.

Methods

Comparative genomics methods were used to study the differences between *C.glabrata*, *C. albicans* and *S. cerevisae.* It involved calling SNPs, assessing the levels of gentetic variations, investigation of recombination rates and strength of purifying selection. What is more, PCR and Sanger sequencing were performed as a validation of computed results and switching of the mating-type.

Conclusions

We determined that *C. glabrata* is highly genetically structured. The existence of some form of sexual cycle is supported by similar patterns of evolutionary constraints in reproduction related genes in *C. glabrata, C. albicans,* and *S. cerevisiae*. Our results are consistent with previous reports of successful mating-type switching in MTL1 from a to alpha and also reveal frequent illegitimate recombination at the other MTL loci.

FEMS7-1804
Pathogens / Pathogenicity - Part II

THE ETIOLOGICAL CHARACTERIZATION OF BACTERIAL MENINGITIS IN CHILDREN IN ASTANA

A. Kushugulova[1], A. Seidullayeva[2], G. Zhaxsylykova[2], D. Bayesheva[2], Y. Chernutskaya[3], A. Brinster[2], S. Zhumadirova[2], B. Turdalina[2], D. Greenberg[4]
[1]National laboratory Astana Nazarbayev University, Laboratory of human microbiome and longevity, Astana, Kazakhstan
[2]JSC Medical University Astana, Department of Pediatric Infectious Diseases, Astana, Kazakhstan
[3]Municipal Children's Infectious Hospital, intensive care unit, Astana, Kazakhstan
[4]The Pediatric Infectious Disease Unit Ben-Gurion University of the Negev Soroka University Medical Center, The Pediatric Infectious Disease Unit Ben-Gurion University, Beer-Sheva, Israel

Backgrounds

Topicality of research is defined the meningococcal disease is characterized by severity, high mortality and disability.

Objectives

To study the bacterial meningitis (BM) etiologic structure and sensitivity to antibiotics in children in Astana.

Methods

A prospective investigation of 30 patients with diagnosis of BM in Astana have been done during 2016, mean age was 36±3month. From it, meningitis of Meningococcal etiology 60%, pneumococcal meningitis 6,6%, meningitis caused by S. enteritidis 3,4%, other cases had unknown etiology.

Conclusions

The BM diagnosis was confirmed from testing the nasopharyngeal smear 30% of cases; in 27% cases the diagnosis was confirmed from investigation of liquor, blood testing in 10% of cases. Bacteriological analysis of nasopharyngeal smear found out that the etiology of the disease was N.meningitidis group A in 13%, in 6,6% cases was related to group B; the W135 group was in 3,46%, Streptococcus pneumoniae was revealed in 6,6 % cases. The analysis of cerebrospinal fluid confirmed the predominant etiologic role of N.meningitidis 17%, in which 10% cases belonged to the group A, in 3,4% cases to B, and to the group W135 3,4% of cases. The bacteriological sowing of liquor found Streptococcus pneumoniae 6.7% of cases and S. enteritidis in 3.4% of cases. N.meningitidis group in 3.4% of cases were identified due to blood analysis. In 56% of patients with Meningococcal disease and 70% of the BM bacteriological analysis results were negative and the diagnosis was based on clinical data. Antibiotics sensitivity analysis of major pathogens of BM showed that the sensitivity of the N.meningitidis had to the following antibiotics: levofloxacin,imipenem,meropenem, penicillin, amoxicillin, cefazoline. Isolated pure cultures Streptococcus pneumoniae demonstrated sensitivityto penicillin, cefazolin, cefuroxime, ceftriaxone, ceftazidime, amoksiklav, oxacillin, makrolidler, imipenem, vancomicin, ciprofloxacin and levofloxacinin 100% of cases and resistance to amixacinum in 50%($p \leq 0,05$).

FEMS7-3154
Pathogens / Pathogenicity - Part II

CHANGES IN METABOLITES AND PROTEINS IN LEAVES OF EUCALYPTUS GRANDIS DURING THE INFECTION OF PUCCINIA PSIDII

M.V. Labate[1], F. Marques[1], T. Cataldi[1], A. Bini[1], I. Budizinski[1], F. Moraes[1], M. Quecine[1], C. Labate[1]
[1]University Of Sao Paulo / Luiz de Queiroz College of Agriculture, Departamento de Genética, Piracicaba, Brazil

Backgrounds

Eucalyptus is constantly exposed to pathogen attack with one of the most threatening diseases being rust caused by the fungus *Puccinia psidii* Winter which is rapidly spreading around the world and was recently described in Australia. To understand the molecular plant-microbe interaction between *E. grandis* and *P. psidii* we developed a model system by isolating the pathogen from a single pustle and select resistant and susceptible plants from half-sib population generated using Brasuz, as the pollen receptor. We performed light and epifluorescence microscopy analyses, identified all of the stages of the fungal development and recognized the moment which the resistant genotype blocks pathogen development. Based on these results we selected six time points to carry out proteomic amd metabolomic analysis of leaves.

Objectives

The aim of this work was to identify the changes in the proteome and metabolome of two contrasting genotypes when exposed to *Puccinia psidii*.

Methods

Qualitative and quantitative (*Label Free Proteomics*) changes of proteins, was evaluated by using an LC-MS/MS mass-spectrometer Synapt G2 HDMS from Waters, in line with a MClass Chromatographer. For metabolomics, a GC-TOF/MS from Leco was used for the identification of primary metabolites and a Q-TOF Ultima API in line with a Acquity chromatographer from Waters.

Conclusions

The results show that the resistant plants notice the presence of the pathogen shortly after being infected, producing immunity related proteins such as *HSP90, ILITYHIA, LRR Kinase, NB-ARC disease resistance protein*. This perception triggers the production of cell wall and oxidative burst proteins, also changing the primary and secondary metabolism. On the other hand, susceptible plants have its metabolism subverted, producing proteins responsible for the cell wall loosening, favoring *P. psidii* nutrient uptake, growth and spread. Metabolite biomarkers, Immune response biomarker molecules and infection signals triggered by *P. psidii* on *E. grandis* are also proposed on this work.

FEMS7-2888
Pathogens / Pathogenicity - Part II

NEW ASPECTS OF TUBERCULOSIS IN PEOPLE WITH HIV: REPORT OF A COHORT OF 92 CASES.

M.E.H. Labdouni[1]
[1]*University of Oran, Scholar of Medecine, Oran, Algeria*

Backgrounds

Tuberculosis is one of the main factors of death among PLHIV, this co-infection has dramatically re-emerged in recent years in our climate. It clothed highly polymorphic aspects; therefore its diagnostic and therapeutic will be very difficult mainly in children.

Objectives

Is to raise the issue of the resurgence of TB in People living with HIV supported in a referent center in western Algeria, its epidemiological, clinical, biological and radiological new trends.

Methods

It was a prospective study during 36 months from the 01st /01/2013 to 31st /12/2015 in identifying and analyzing cases of TB / HIV co infection.

Conclusions

Co-infection TB / HIV reemerged in recent years in our patients taking a more invasive appearance with frequency and multifocal forms of liver disease thus causing relatively a high mortality.

FEMS7-0224
Pathogens / Pathogenicity - Part II

ANTIBIOTIC-INDUCED STRINGENT RESPONSE IN ENTEROCOCCUS FAECALIS EVALUATED USING AN IN VITRO MODEL OF A CATHETERISED URINARY TRACT

S. Lang[1], R. Adeigbe[1]
[1]Glasgow Caledonian University, Department of Life Sciences, Glasgow, United Kingdom

Backgrounds

Enterococci exposed to environmental stresses of the urinary tract may undergo physiological changes leading to an overall switch from the expression of genes required for growth to those that will aid survival; a phenomenon known as the stringent response (SR).

Objectives

This study assessed the ability of antibiotics used in the treatment of *E.faecalis*-related catheter-associated urinary tract infection (CAUTI) to induce the SR and evaluate the impact of the induced-SR on virulence.

Methods

Gene expression of four clinical enterococcal strains was studied using real-time PCR during mid-exponential phase of growth in planktonic cells and biofilms in pooled human urine. Cultures exposed to the antibiotics trimethoprim, nitrofurantoin, ciprofloxacin and fosfomycin were assessed for induction of the SR (*relA* and *relQ*-markers of induction), changes in the expression of stress-related genes (*gls24, eep*), adhesins (*ebpA, ace, esp, efaA*), toxins (*cyl, hyl*), and proteases (*spr, enl, gelE*).

Conclusions

Exposure to trimethoprim or nitrofurantoin triggered the SR, during which all the adhesin, toxin, protease (except *spr* in one strain) and stress-related genes investigated showed significant up-regulation (≥2-fold change in gene expression). Neither ciprofloxacin nor fosfomycin induced the SR. Exposure to a combination of nitrofurantoin or trimethoprim with either fosfomycin or ciprofloxacin prevented the induction of the SR and the associated increase in the expression of virulence genes.

Under conditions mimicking CAUTI, treatment with commonly used antibiotics has the potential to increase the virulence of *E.faecalis*. Combination therapy however, has the potential to not only improve the treatment of these recalcitrant infections, but inhibit stress-responses and associated increased pathogenicity.

FEMS7-0924
Pathogens / Pathogenicity - Part II

EFFECTS OF SUB-MICS OF COLISTIN ON ACINETOBACTER BAUMANNII BIOFILMS

M. Lázaro-Díez[1], I. CHAPARTEGUI-GONZÁLEZ[2], Z. Bravo-del Hoyo[1], T. Navascués-Lejarza[1], A. Ocampo-Sosa[1], J. Calvo[3], L. Martínez-Martínez[3], J. Ramos-Vivas[1]
[1]IDIVAL, Microbiology, Santander, Spain
[2]IDIVAL, Microbiolgy, Santander, Spain
[3]Hospital Universitario Marqués de Valdecilla HUMV, Microbiology, Santander, Spain

Backgrounds

Acinetobacter spp. are recognized as emerging nosocomial pathogens and a primary cause of Gram negative infections in many parts of the world. These bacteria, mainly *A. baumannii*, are particularly difficult to trait due to its propensity to develop resistances to many groups of antibiotics.

In *A. baumannii,* biofilm is increasingly recognized as an important bacterial virulence trait that encourages antibiotic resistance. Therefore, evaluation of antimicrobial agents against *Acinetobacter* should include an assessment of their ability to interfere with biofilms.

Objectives

The aim of this study was to investigate the responses of *Acinetobacter baumannii* biofilms to sub-inhibitory concentrations of colistin (one of the last choices to trait *A. baumannii* infections).

Methods

Ten *A. baumannii* strains were used in this study. Biofilm formation and disruption of preformed biofilms in presence of different concentrations of colistin were quantitatively assessed by crystal violet staining. We have also investigated the morphology of some of these strains challenged *in vitro* with sub-MICs by TEM. Biofilm cell viability was determined with Live/Dead staining using CLSM.

Conclusions

MICs of colistin ranged from 1 to 64 µg/ml. Four of 10 strains tested were designated as strong-biofilms formers. In these strains, our results showed that colistin affected the biofilm formation at MIC. However, 50% strains colistin was able to increases biofilm formation at certain sub-MICs. In some strains, colistin was unable to disrupt preformed biofilms. Electron micrographs taken from planktonic cells showed an array of deformations and structural changes in bacteria after exposure to this drug.

FEMS7-2196
Pathogens / Pathogenicity - Part II

A NEW ENZYME WITH DUAL-FUNCTION FRUCTOSE/SEDOHEPTULOSE BISPHOSPHATASE SUSTAINS GLUCONEOGENESIS IN BRUCELLA SUIS BIOVAR 5

L. Lázaro[1], M.J. de Miguel[2], F. Baldin[3], E. Van Schaftingen[3], R. Conde-Álvarez[1], P.M. Muñuz[2], M. Iriarte[1], J.J. Letesson[4], A. Zúñiga-Ripa[1], I. Moriyón[1]
[1]Universidad de Navarra, Instituto de Salud Tropical. Microbiología y Parasitología, Pamplona, Spain
[2]Centro de Investigación y Tecnología Agroalimentaria CITA, Unidad de Sanidad Animal, Zaragoza, Spain
[3]Université Catholique de Louvain, Welbio and de Duve Institute, Brussels, Belgium
[4]UNAmur, Research Unit in Biology of Microorganisms Biology, Namur, Belgium

Backgrounds

Bacteria of the genus *Brucella* are facultative intracellular parasites causing brucellosis, a worldwide-extended zoonosis. The pathogenicity of these bacteria resides in their ability to adjust their metabolism to the nutrients available in the intracellular niche. Recently, we showed that a *B. suis* biovar 5 double mutant in the phosphoenolpyruvate carboxykinase (PckA) and the pyruvate phosphate dikinase (PpdK), two anabolic enzymes bridging the TCA cycle and the gluconeogenic pathway, is attenuated. Unexpectedly, a double mutant in the two genes (*fbp*, *glpX*) encoding a fructose-1,6-bisphosphatase (FBPase) was able to grow under gluconeogenic carbon sources.

Objectives

Since FBPases are essential for gluconeogenesis, this observation suggested that *B. suis* 5 remains gluconeogenically competent in the absence of Fbp and GlpX. The aim of this work was to identify the third FBPase or the metabolic bypass that sustains gluconeogenesis when Fbp and GlpX are absent.

Methods

Bibliographic and genomic analyses allowed us to identify the phosphatase Gpm. We constructed a triple mutant *fbp-glpX-gpm* and we tested the capability of the mutant to grow on gluconeogenic substrates. Finally, we studied the infection kinetics of the mutant in mice. Moreover, we expressed, purified and characterized Gpm.

Conclusions

The mutant lacking the three FBPases was not able to grow on gluconeogenic substrates and was attenuated in the mouse model, confirming that gluconeogenesis is essential during infection. Moreover, characterization of Gpm showed that i), it has dual-function fructose-1,6/sedoheptulose-1,7-bisphosphatase; ii), it does not require a metal cofactor and iii), it does not belong to any of the five types of FBPases.

FEMS7-1360
Pathogens / Pathogenicity - Part II

VISUALIZATION OF THE ROLE OF HOST HEME ON THE VIRULENCE OF THE HEME AUXOTROPH STREPTOCOCCUS AGALACTIAE

D. Lechardeur[1], L. Joubert[1], J.B. Dagieu[1], F. Annabelle[2], A. Derré-Bobillot[1], E. Borezée-Durant[1], I. Fleurot[3], A. Gruss[1]
[1]INRA-Institut National de la Recherche Agronomique, Micalis, Jouy en Josas, France
[2]Bel, Fromagerie Bel, Vendôme, France
[3]INRA-Institut National de la Recherche Agronomique, Unité ISP, Nouzilly, France

Backgrounds

Heme is essential for several cellular key functions but is also toxic. Whereas most bacterial pathogens utilize heme as a metabolic cofactor and iron source, the impact of host heme during bacterial infection remains elusive. The opportunist pathogen *Streptococcus agalactiae* does not synthesize heme but still uses it to activate a respiration metabolism. Concomitantly, heme toxicity is mainly controlled by the HrtBA efflux transporter.

Objectives

Here we investigate how *S. agalactiae* manages heme toxicity *versus* benefits in the living host.

Methods

We used bioluminescent bacteria and heme-responsive reporters for *in vivo* imaging,

Conclusions

We show that the capacity of *S. agalactiae* to overcome heme toxicity is required for successful infection, particularly in blood-rich organs. Host heme is simultaneously required, as visualized by a generalized infection defect of a respiration-negative mutant. In *S. agalactiae*, HrtBA expression responds to an intracellular heme signal *via* activation of the two-component system HssRS. A *hssRS* promoter-driven intracellular luminescent heme sensor was designed to identify host compartments that supply *S. agalactiae* with heme. *S. agalactiae* acquires heme in heart, kidneys, and liver, but not in the brain. We conclude that the *S. agalactiae* response to heme is organ-dependent, and its efflux may be particularly relevant in late stages of infection.

FEMS7-0645
Pathogens / Pathogenicity - Part II

GENOME-WIDE AND COMPARATIVE ANALYSIS OF SECRETED AND TRANSMEMBRANE PROTEINS IN BURKHOLDERIA SPECIES

H.H. Lee[1], N. Thao Thi[1], N. Kim[1], I. Kim[1], J. Park[1], I. Park[2], Y.S. Seo[1]
[1]Pusan National University, Microbiology, Busan, Republic of Korea
[2]Youngsan University, Oriental Culinary, Busan, Republic of Korea

Backgrounds

The *Burkholderia* genus is composed of not only important pathogens infecting plants, animals and humans but also non-pathogenic bacteria like endophytes. Secreted and transmembrane proteins of the *Burkholderia* genus especially play an essential role these various interactions.

Objectives

We tried to identify and compare the secreted proteins of six representative *Burkholderia* species consisting of plant pathogens (*B. glumae* BGR1, *B. gladioli* BSR3), human pathogens (*B. pseudomallei* K96243, *B. cepacia* LO6), and endophytes (*Burkholderia* sp. KJ006, *B. phytofirmans* PsJN) through the genome-scale computational identification.

Methods

The whole genome sequences of the six representative *Burkholderia* species were downloaded from the RefSeq database at the NCBI website. The secreted proteins were predicted and analyzed by SignalP, TatP, LipoP and TMHMM programs and the SecretomeP, EffectiveT3 and T3SS_prediction servers.

Conclusions

We found that the proportions of putative classically secreted proteins (CSPs) and transmembrane (TM) proteins of all species were significantly high up to approximately 20%, besides the lower proportions of putative type 3 non-classically secreted proteins (T3NCSPs) (~10%) and unclassified non-classically secreted proteins (NCSPs) (~5%). The fractions of TM proteins were separated but the distribution of the number of TM domains was conserved. In addition, the protein size distribution of the secreted protein groups was conserved among the species and species-specific differences in the functional characteristics of these proteins in CSPs, T3NCSPs, and unclassified NCSPs. This study could provide new insights into the relationship among plant-pathogenic, human-pathogenic, and endophytic bacteria.

FEMS7-0190
Pathogens / Pathogenicity - Part II

QUORUM SENSING-DEPENDENT POST-SECRETIONAL ACTIVATION OF PROTEASE IV IN PSEUDOMONAS AERUGINOSA

J.H. Lee[1], J. Oh[1], X.H. Li[1], S.K. Kim[1]
[1]Pusan National University, Department of Pharmacy, Busan, Republic of Korea

Backgrounds

Protease IV (PIV), a key virulence factor of *Pseudomonas aeruginosa* is a secreted lysyl-endopeptidase whose expression is induced by quorum sensing (QS), a cell-density dependent cell to cell communication system. QS is very important in infection of pathogens.

Objectives

We found that PIV expressed in QS mutant has severe reduction of activity in culture supernatant (CS), even though it is overexpressed to high level. We intended to elucidate the underlying mechanism in this post-secretional activation of PIV by QS.

Methods

The PIV purified from the CS of QS mutant (M-PIV) had much lower activity than the PIV purified from the CS of wild type (P-PIV), although two PIVs has no difference. We found that the propeptide cleaved from prepro-PIV was always co-purified with M-PIV, but never with P-PIV. Since the activity of M-PIV could be restored by the addition of the CS prepared from a QS-positive and PIV-deficient strain, we hypothesized that the propeptide binds to and inhibits PIV, and is degraded to activate PIV by a QS-dependent factor. In fact, the CS of the QS-positive strain was able to degrade the propeptide. Since the responsible factor should be a QS-dependently expressed extracellular protease, we tested QS-dependent proteases of *P. aeruginosa* and found that LasB can degrade the propeptide and activate M-PIV. We purified the propeptide and confirmed the propeptide can bind to and inhibit PIV.

Conclusions

We suggest that PIV is post-secretionally activated through the extracellular degradation of the propeptide by LasB, a QS-dependent protease.

FEMS7-1716
Pathogens / Pathogenicity - Part II

IDENTIFICATION OF NOVEL MYCOBACTERIUM TUBERCULOSIS ANTIGENS FOR IMMUNODIAGNOSIS BY A BIOINFORMATIC ANALYSIS

J.M. Ha[1], W.G. Yoo[2], S.H. Kim[1], M.S. Park[1], S. Lee[1]
[1]Korea National Institute of Health, Division of Tuberculosis and Bacterial Respiratory Infections, Cheongju, Republic of Korea
[2]Chung-Ang University College of Medicine, Department of Medical Environmental Biology, Seoul, Republic of Korea

Backgrounds

New biomarkers derived from *Mycobacterium tuberculosis* have been investigated for the accurate diagnosis of tuberculosis (TB). Especially, multifaceted approaches are required for finding diagnostic antigens related to the latent TB infection.

Objectives

To investigate new biomarkers for immunodiagnosis of tuberculosis, we performed an extensive analysis of the antigens and epitopes of *M. tuberculosis* K strain, coupled bioinformatics with reverse vaccinology.

Methods

Protein sequences (4,146 amino acids) of *M. tuberculosis* K strain were retrieved from National Center for Biotechnology and Information database. Sequences of BCG strains, non-tuberculoid *Mycobacteria*, and other tuberculoid *Mycobacteria* were extracted from MtbVeb. *M. tuberculosis* specific proteins were identified, comparing with sequences of BCG strains and other *Mycobacteria* spp. Protein localization was predicted by using 7-sub-cellular predictors. Antigen and epitope candidates having interferon-gamma secreting major histocompatibility complex class II peptides were predicted by using Immuno Epitope Database and Analysis Resources and IFNepitope.

Conclusions

One hundred and twenty eight unique proteins including 3 *M. tuberculosis* latency antigens were identified, which did not have any homology with those of 5 BCG vaccine strains and 34 species of non-tuberculoid *Mycobacteria*. Thirty seven secretory proteins were selected and then 17 secretory antigen candidates were predicted to induce IFN-γ secretion. Comparing our candidates with sequences of 1,036 antigens and 7,683 epitopes extracted from IEDB, 12 novel antigens were finally selected. And 4 to 16 sequences of human T cell epitopes were predicted for each antigen. Our results will provide new antigenic targets for the development of advanced immunodiagnostic tools.

FEMS7-0487
Pathogens / Pathogenicity - Part II

INVERSE AUTOTRANSPORT: THE MECHANISM OF TYPE VE SECRETION

J.C. Leo[1], *P. Oberhettinger*[2], *M. Schuetz*[2], *D. Linke*[1]
[1]*University of Oslo, Department of Biosciences, Oslo, Norway*
[2]*University Clinics Tuebingen, Institute for Medical Microbiology and Hygiene, Tuebingen, Germany*

Backgrounds

Intimin and invasin are adhesins and central virulence factors of enterohaemorrhagic *E. coli* enteropathogenic *Yersiniae*, respectively. These proteins are prototypes of a large family of adhesins and represent a previously unrecognized autotransporter secretion system, termed type Ve secretion. Classical (type Va) autotransporters consist of a C-terminal β-barrel domain, or translocation unit, and an N-terminal extracellular region or passenger. According to the prevailing model of classical autotransport, the translocation unit exports the passenger C-terminus first, i.e. through a hairpin intermediate.

Objectives

In intimin and invasin, the domain order is reversed compared to classical autotransporters. We aimed to establish the topology and characterize the secretion process of these proteins.

Methods

We investigated the topology of intimin and invasin by introducing epitope tags at selected sites within the protein. Furthermore, we examined whether the passenger is transported through the pore of the translocator unit. We discovered that the insertion of an epitope tag into the membrane-proximal region of the Intimin passenger leads to a non-adherent phenotype, and closer examination showed that the passenger of this variant was still located in the periplasm despite the β-barrel domain being inserted into the outer membrane and correctly folded. This insertion disrupts folding of the proximal immunoglobulin-like domain.

Conclusions

Our results confirm the predicted topology of the translocation units of intimin and invasin and demonstrate that their passengers are exported via a hairpin intermediate in an N-to-C direction. Furthermore, passenger secretion is driven by protein folding. These findings have prompted us to use the moniker ‘inverse autotransporter’ for these proteins.

FEMS7-2531
Pathogens / Pathogenicity - Part II

A NOVEL CONJUGATIVE ELEMENT COMPOSED OF TN5801 AND A NEW TRANSPOSON: TRANSFER DYNAMICS BETWEEN ENTEROCOCCUS FAECALIS POPULATIONS

R. León-Sampedro[1], Á. San Millán[1], C. Novais[2], L. Peixe[2], F. Baquero[1], T.M. Coque[1]
[1]Instituto Ramón y Cajal de Investigación Sanitaria, Molecular Microbiology, Madrid, Spain
[2]UCIBIO/REQUIMTE - University of Porto, Microbiology Laboratory, Porto, Portugal

Backgrounds

Conjugative-transposon (CTn) Tn*5801*, a Tn*916-tet*(M) member conferring resistance to tetracycline, was detected in disparate Firmicutes genus, mainly Enterococcus. However, dynamics of transfer events still remains unexplored.

Objectives

To analyze the mechanism of transfer of Tn*5801* and the impact of acquiring this CTn in the bacterial fitness of *Enterococcus faecalis (Efs).*

Methods

Tn*5801* was transferred by filter mating using a clinical strain as donor, and different *Efs* laboratory recipient strains: JH2-2 (with a Tn*5801*-Δ*tet*M), OG1RF and OG1SSp. Secondary filter mating assays were performed using *Efs*-JH2-2 transconjugants as donors. Mating plates were supplement with 10mg/L of tetracycline. Whole genome sequencing (Illumina-HiSeq-2500) of primary JH2-2 and secondary OG1SSp transconjugants was performed to analyze conjugative events recombinations. Growth rate was estimated in a Synergy HTX Plate Reader in the absence and presence of sub-inhibitory tetracycline concentrations (0,5-2mg/L).

Conclusions

We describe the transmission of Tn*5801* among *Efs* using clonal backgrounds with/without a pre-existing Tn*5801* copy. Transference of a novel 50kb composite element-50CE (Tn-30kb+Tn*5801*-20kb) occurred in a in specific 11bp sequence at the chromosome of primary JH2-2 filter mating and secondary OG1SSp transconjugants carrying one or two 50CE copies. Transconjugants harboring two 50CE copies showed higher values of $MIC_{Tetracycline}$ and V_{max} of the growth curves than those with a single 50CE copy (24-48 vs 96vs1mg/L and 4.28 vs 5.97, respectively). The data suggest that widespread use of tetracycline might contribute for Tn916-elements transfer and bacterial fitness in tetracycline selective environments, which could facilitate the persistence and amplification of some pathogenic clones and mobile genetic elements.

FEMS7-1696
Pathogens / Pathogenicity - Part II

UNRAVELING HOST CELLULAR FUNCTIONS EMPLOYED BY MRSA DURING INTRACELLULAR SURVIVAL

M. Letek[1], N. Bravo[1], V. Behrends[1]
[1]University of Roehampton, Life Sciences, London, United Kingdom

Backgrounds

As a facultative intracellular pathogen, Methicillin-resistant *Staphylococcus aureus* (MRSA) is able to invade and proliferate within mammalian cells, including professional and non-professional phagocytes. This intracellular survival may be used by the pathogen to evade host immune responses as well as exposure to antibiotics.

Objectives

While most studies have focused on the virulence factors of MRSA, host molecular factors exploited by the pathogen during intracellular survival remain mostly unknown. Our research aims to identify novel host-directed therapies against MRSA by studying the host molecular and metabolic factors exploited by the pathogen during infection.

Methods

We have employed shRNA-based genome-wide screens to identify novel host factors essential for the intracellular survival of MRSA. This has been complemented with a metabolomics approach based on Gas Chromatography Mass Spectrometry, in combination with stable isotope-labelling.

Conclusions

By employing shRNA screens, subsequent bioinformatics and *in silico* analysis, we have identified several host-cell genes that could be potentially involved in *S. aureus* pathogenesis. Our results show that silencing certain genes in HeLa cells increases host cell viability after MRSA infection whereas bacterial survival is significantly reduced. This suggests that *S. aureus* is able to modulate different molecular pathways of the host cell for its intracellular survival.

On the other hand, our metabolomics approaches indicate that the Tricarboxylic cycle and lower glycolysis pathway are affected by the presence of MRSA within the cell.

In our conference contribution, we will discuss these findings, which provide a better understanding of the MRSA-host cell interactions and may lead to novel treatments against this versatile pathogen.

FEMS7-0652
Pathogens / Pathogenicity - Part II

P-113-DERIVED ANTIMICROBIAL PEPTIDES AGAINST CANDIDA ALBICANS

G.Y. Lin[1], W.C. Cheng[2], C.Y. Lan[1]
[1]National Tsing Hua University,
Institute of Molecular and Cellular Biology and Department of Life Sciences, Hsinchu, Taiwan R.O.C.
[2]General Biologicals Corporation, Department of Research & Development, Hsinchu, Taiwan R.O.C.

Backgrounds

Candida albicans is a commensal organism that is commonly found on the mucosal surfaces, nails and skin. However, *C. albicans* is also an opportunistic fungal pathogen that can infect immunocompromised patients and cause life-threatening infections. One of the reasons for the mortality is the emergence of antifungal-drug resistance in *C*. *albicans*. Therefore, it is important to develop new antifungal drugs.

Objectives

In this study, we studied the mechanisms of action for P-113-derived peptides to kill *C. albicans.* Particularly, the functions of these antimicrobial peptides on the cell wall of *C*. *albicans*.

Methods

We used a range of cell wall-defective mutants of *C. albicans* to investigate interactions between the fungicidal ability of the cationic antimicrobial peptides and the cell wall. In addition, we performed binding assay to determine the interaction between various cell wall components and the antimicrobial peptides.

Conclusions

We found a decreased binding and susceptibility of the antimicrobial peptides in different *C. albicans* mutants. The results showed that antimicrobial peptides need to recognize molecular patterns on the pathogens surface to enhance their activity and specificity against *C*. *albicans*.

FEMS7-0651
Pathogens / Pathogenicity - Part II

CHARACTERIZATION OF BIOFILM PRODUCTION IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII AND THE EFFECTS OF CHEMICAL COMPOUNDS ON BIOFILM FORMATION

M.F. Lin[1], Y.Y. Lin[2], C.Y. Lan[2]
[1]National Taiwan University Hospital Chu-Tung Branch, Department of Medicine, Hsinchu, Taiwan R.O.C.
[2]National Tsing Hua University, Institute of Molecular and Cellular Biology, Hsinchu, Taiwan R.O.C.

Backgrounds

Acinetobacter baumannii, an emerging important pathogen of nosocomial infection, is infamous for its ability to form biofilms. Biofilm formation increases the survival rate of *A. baumannii* on dry surfaces and may contribute to its persistence in the hospital environment, increasing the probability of causing nosocomial infections and outbreaks.

Objectives

This study was undertaken to characterize biofilm production of the clinical isolates of *A. baumannii* and the effects of chemical compounds, especially antibiotics, on biofilm formation.

Methods

We first determined biofilm formation of the wild type, mutant strains and clinical isolates of *A. baumannii* by XTT assay. The biofilm formation was also evaluated using scanning electron microscopy and confocal laser scanning microscopy. Moreover, the influence of amikacin, imipenem, colistin, tigecycline, LL-37 and tannic acid on biofilm formation was examined. Finally, the minimal planktonic and biofilm inhibitory concentrations of the above antibiotics to *A. baumannii* were determined.

Conclusions

The ability of biofilm formation in *A. baumannii* was various in different strains, involved many genes and could be influenced by many chemical compounds. The minimal biofilm inhibitory concentrations of all the tested antibiotics were increased for both the wild type and the clinical isolate of multidrug resistant *A. baumannii* VGH2.

FEMS7-2293
Pathogens / Pathogenicity - Part II

RESISTANCE GENE TRANSFER: INDUCTION OF TRANSDUCING PHAGE BY SUB-INHIBITORY CONCENTRATIONS OF ANTIMICROBIALS IS NOT CORRELATED TO INDUCTION OF LYTIC PHAGE

K. Stanczak-Mrozek[1], K. Laing[1], J. Lindsay[1]
[1]St George's- University of London, Infection and Immunity, London, United Kingdom

Backgrounds

Horizontal gene transfer (HGT) of antimicrobial resistance genes (AMR) genes between clinical isolates via transduction is poorly understood. MRSA are opportunistic pathogens resistant to all classes of antimicrobial agents but currently no strains are fully drug resistant. AMR gene transfer between *S. aureus* isolates is predominantly due to generalized transduction via endogenous bacteriophage, and recent studies have suggested transfer is elevated during host colonization.

Objectives

The aim was to investigate whether exposure to sub-MIC concentrations of antimicrobials triggers bacteriophage induction and/or increased efficiency of AMR gene transfer.

Methods

Isolates from MRSA carriers were exposed to nine antimicrobials and supernatants compared for lytic phage particles and ability to transfer an AMR gene. A new technology, droplet digital PCR (ddPCR) measured the concentration of genes in phage particles.

Conclusions

All antibiotics tested induced lytic phage and AMR gene transduction, although the ratio of transducing particles to lytic particles differed substantially for each antibiotic. Mupirocin induced the highest ratio of transfer versus lytic particles. Gentamicin and novobiocin reduced UV-induced AMR transduction. The genes carried in phage particles correlated with AMR transfer or lytic particle activity, suggesting antimicrobials influence which DNA sequences are packaged into phage particles.

Sub-inhibitory antibiotics induce AMR gene transfer between clinical MRSA, while combination therapy with an inhibiting antibiotic could potentially alter AMR gene packaging into phage particles, reducing AMR transfer. In a continually evolving environment, pathogens have an advantage if they can transfer DNA while lowering the risk of lytic death.

FEMS7-0335
Pathogens / Pathogenicity - Part II

SOLID-STATE NMR STUDIES OF YADA (YERSINIA ADHESIN A): FROM AUTOTRANSPORT TO DRUG SCREENING

N. Chauhan[1], M. Orwick-Rydmark[1], D. Linke[1]
[1]University of Oslo, Biosciences, Oslo, Norway

Backgrounds

Yersinia enterocolitica is a Gram-negative enteric pathogen that can cause diarrhea, and complications such as sepsis or reactive arthritis when passing from the gut into the blood stream and beyond. Adhesins that allow the initial adhesion in the gut are important pathogenicity factors for tissue invasion. One major adhesin of *Y. enterocolitica* is YadA, which binds to collagen of the extracellular matrix. It is the prototype of type Vc secretion systems, so-called autotransporters that form a pore in the bacterial outer membrane to transport their extracellular domain to the cell surface independent of external energy sources such as ATP.

Objectives

Understanding the autotransport process and the binding mode of this adhesin will help us to find small-molecule drugs that can inhibit initial adhesion to gut tissue.

Methods

We use a combination of solid-state NMR techniques and targeted mutagenesis to understand the autotransport process. We have recently published a full ssNMR structure of the YadA membrane anchor, and are now studying mutants stalled in the autotransport process. I will present structural details of such an autotransport intermediate, and will discuss possibilities how this conserved mechanism could be targeted with small-molecule drugs.

Conclusions

I will present structural details of such an autotransport intermediate, and will discuss possibilities how this conserved mechanism could be targeted with small-molecule drugs.

FEMS7-2147
Pathogens / Pathogenicity - Part II

MYCOBACTERIUM ABSCESSUS AND THE HUNT OF THE CELL WALL COMPONENT RESPONSIBLE FOR CORDING

M. Llorens-Fons[1], M. Pérez-Trujillo[2], E. Julián[1], F. Alcaide[3], M. Luquín[1]
[1]Universitat Autònoma de Barcelona, Departament de Genètica i Microbiologia, Bellaterra, Spain
[2]Universitat Autònoma de Barcelona, Servei de Ressonància Magnètica Nuclear, Bellaterra, Spain
[3]Hospital Universitari de Bellvitge-IDIBELL, Departament de Microbiologia, L'hospitalet de Llobregat, Spain

Backgrounds

Many mycobacteria species have two morphotypes, the rough (R) that form winkled colonies and the smooth (S) that form even colonies. R morhotypes are more pathogenic than S morphotypes, which seems to be related with the organization of the bacilli. R morphotype bacilli dispose in parallel forming cords, in contrast, S morhotypes bacilli do not present any organization.

As cording is an important virulence factor, many studies have been performed in order to discover a compound responsible for the cord formation. In 1956 the trehalose dimycolate (TDM) from *Mycobacterium tuberculosis* was described as the “cord factor”. However, years later it has been detected TDM in no-cording mycobacteria.

Objectives

Our objective is to find the compound responsible for cording in *Mycobacterium absessus*, using this bacteria as a model of *M. tuberculosis*.

Methods

Different strains of *M. abscessus* were grown in Middlebrook 7H9 broth in order to obtain biopellicles in the surface of the media. An extraction of the superficial lipids was performed on the biopellicles using petroleum ether. Then, the delipidated biopellicles were observed under optical microscope and compared to the untreated biopellicles. The petroleum ether extracts were analysed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

Conclusions

When treated with petroleum ether R morphotype bacteria lost its organization, cords seemed to be fraying. On the extracts, a lipidic compound was found only in R morphotypes, which seems to indicate a relation between this cell wall component and the cording.

FEMS7-1819
Pathogens / Pathogenicity - Part II

BISTABLE EXPRESSION OF RELEVANT GENES FOR PSEUDOMONAS SYRINGAE VIRULENCE

N. López-Pagán[1], J.S. Rufián[1], D. López-Márquez[1], J. Ruiz-Albert[1], C.R. Beuzón[1]
[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" IHSM-UMA-CSIC, Departamento de Biología Celular- Genética y Fisiología. Facultad de Ciencias. Universidad de Mála ga. 29071, Málaga, Spain

Backgrounds

Heterogeneity or phenotypic variation has been known to take place in microbial clonal populations for decades. Under certain regulatory circuits, heterogeneity in gene expression can be enhanced leading to a bimodal expression profile in homogeneous environments. This process is known as bistability. The relevance of these processes has been demonstrated in *Salmonella enterica* and other human pathogen in the establishment of antibiotic persistence, and it has been shown to affect virulence genes, and to be linked to the establishment of chronic persistence. Nevertheless, little is known about the occurrence or impact of these processes in the adaptation of bacteria to non-animal host.

Objectives

To address the question of whether there is phenotypic heterogeneity in the expression of genes relevant for adaptation to a non-animal host of the model plant pathogen *Pseudomonas syringae*.

Methods

Transcriptional chromosome-located fusion to reporter genes encoding fluorescent protein were constructed in *P. syringae* pv. *phaseolicola*. Genes selected were several encoding different elements of a type III secretion system (T3SS) and flagellin, since motility has been reported as counter-regulated with the T3SS, Expression from these genes was analyzed using single-cell analysis methods, such as flow cytometry and fluorescent microscopy.

Conclusions

We recently showed expression of T3SS genes is phenotypically heterogeneous in planta and becomes bistable under certain laboratory conditions through the action of a double regulatory loop on the transcriptional activator HrpL. We present here single cell analyses of the gene encoding flagellin, as well as its responses to different regulatory proteins.

FEMS7-0500
Pathogens / Pathogenicity - Part II

A NOVEL BIARYL AMIDE COMPOUND WITH INHIBITORY ACTIVITY AGAINST CANDIDA ALBICANS FILAMENTATION AND BIOFILM FORMATION AS AN ANTI-VIRULENCE AGENT FOR THE TREATMENT OF CANDIDIASIS

J. Romo[1], C. Pierce[2], A. Lazzell[1], S. Saville[1], J. Lopez-Ribot[1]
[1]The University of Texas at San Antonio, Biology, San Antonio, USA
[2]University of the Incarnate Word, Biology, San Antonio, USA

Backgrounds

Candida albicans is the most common fungal pathogen. The yeast-to-hyphae transition and biofilm formation represent two main virulence factors in *C. albicans.*

Objectives

Targeting functions essential for virulence constitutes an attractive, yet clinically unexploited alternative for the development of new antifungal agents.

Methods

We have performed high content screenings, with a total of 30,000 small molecule compounds from Chembridge's DIVERSet™ chemical library, in search for inhibitors of *C. albicans* filamentation and biofilm formation, and identified several hit compounds. Of these, a series of compounds with a common biaryl amide core demonstrated potent inhibitory activity against both filamentation and biofilm formation at low micromolar concentrations. The leading compound of this series was able to prevent filamentation under all liquid and solid media conditions tested, suggesting that it impacts a core component of the cellular machinery mediating hypha formation under different environmental conditions. The compound demonstrated *in vivo* activity in three different clinically-relevant murine models of invasive candidiasis, oral candidiasis and catheter-related candidemia. This compound displayed activity against a panel of azole resistant clinical isolates. RNA-Sequencing data revealed that treatment with this compound results in differential expression of key filamentation and biofilm genes. Current efforts are aimed at identifying its target at the molecular level. We have also embarked in a medicinal chemistry campaign to identify analogues with improved pharmacodynamic and pharmacokinetic properties.

Conclusions

Based on its *in vitro* and *in vivo* activities, this leading compound represents a promising candidate for the development of novel anti-virulence strategies against *C. albicans* infections.

FEMS7-1735
Pathogens / Pathogenicity - Part II

PHENOTYPIC CHARACTERIZATION OF BERGEYELLA FROM THE NASAL MICROBIOTA OF SWINE

M. Lorenzo de Arriba[1], S. Lopez-Serrano[1], N. Galofre-Mila[1], V. Aragon[1]
[1]Centre de Recerca en Sanitat Animal CReSA-IRTA, Endemic diseases, Bellaterra, Spain

Backgrounds

Bacterial diseases in livestock are commonly treated with antimicrobials. However excessive use of antimicrobials has favoured the emergence of resistances to these drugs, in consequence reducing their effectiveness. Inappropriate administration of antimicrobials also produces dysbiosis of the microbiota, which can facilitate colonization by pathogens. In swine, nasal microbiota has been shown to have an effect on the predisposition to Glässer's disease, caused by *Haemophilus parasuis*. Among others, one of the most abundant genus in the nasal cavities of piglets at weaning was *Bergeyella*.

Objectives

The objective of this work was to characterize nasal isolates of *Bergeyella* and evaluate their virulence potential.

Methods

Nasal swabs from 3-4 week-old piglets from 8 commercial farms of domestic pigs and 1 farm of wild boars were cultured under aerobic conditions. Isolates were identified by 16S rRNA gene sequencing and later genotyped to assess different strains. *Bergeyella zoohelcum* and *Bergeyella porcorum* were identified within the 11 different strains. In general *Bergeyella* isolates showed resistance to serum complement and phagocytosis, showed poor biofilm formation capacity, and were able to adhere to epithelial cells. Maneval staining revealed that *Bergeyella* strains seemed to have capsule. Multiresistance to antimicrobials was found in 10 of the 11 strains, including one strain isolated from wild boar with no history of antimicrobial use.

Conclusions

In conclusion, *Bergeyella* strains from the nasal cavities of piglets showed some *in vitro* features indicative of virulence potential. Further studies are needed to identify the role of *Bergeyella* in disease and within the nasal microbiota of swine.

FEMS7-2886
Pathogens / Pathogenicity - Part II

XYLELLA FASTIDIOSA, THE HIDDEN THREAT FOR THE MEDITERRANEAN AGRICULTURE

M.M. López[1], D. Olmo[2], A. Nieto[2], A. Monterde[1], M. Montes-Borrego[3], J. Peñalver[1], K. Ouertani[1], C. Morente[1], I. Navarro[1], M.T. Gorris[1], E. Marco-Noales[1], B.B. Landa[3]
[1]Instituto Valenciano de Investigaciones Agrarias, Bacteriología, Moncada Valencia, Spain
[2]Gobierno Islas Baleares, Sanidad Vegetal, Palma de Mallorca, Spain
[3]Consejo Superior de Investigaciones Científicas, Instituto de Agricultura Sostenible, Córdoba, Spain

Backgrounds

Xylella fastidiosa is a xylem limited bacteria, considered a quarantine organism. Originally circumscribed to America, it has been recently identified in Italy, France, Germany and Spain. Its economic importance is due to the large number of plant hosts, the severe symptoms induced, the long list of vectors and its difficult control. This pathogen represents a threat not only for European agricultural crops, but also for landscape trees and ornamentals.

Objectives

An update of the diseases caused by *X. fastidiosa*, new information about its biology and epidemiology in Europe and the role of accurate diagnostic protocols.

Methods

Following the identification in 2013 of *X. fastidiosa* subsp. *pauca* in southern Italy, rapid emergency measures have been implemented in the EU for its prevention. Available information suggests that the Italian strain could have been introduced with ornamental plants imported from Costa Rica. In addition, in Spain, the first detection in Mallorca island, in October 2016 using the EPPO protocol of diagnostic, was immediately followed by new detections in the nearby Ibiza island. Later on, the pathogen has been detected in 92 points in olive, almond, stone fruit trees and many ornamentals. There are several subspecies of *X. fastidiosa* involved and vectors are under study.

Conclusions

These two examples demonstrate that the global market could lead to dissemination of quarantine organisms. The prevention of this pathogen requires further investigations, as well as intensive surveys and accurate analytical methods (PonTE and XF-ACTORS projects financed by the H2020 Program of the European Union).

FEMS7-2745
Pathogens / Pathogenicity - Part II

DETECTION OF BIOFILM FORMATION AMONG THE CLINICAL ISOLATES OF METYCILIN-RESISTANT STAPHYLOCOCCUS AUREUS

N. Łubowska[1], *L. Piechowicz*[1]
[1]*Medical University of Gdansk, Medical Microbiology, Gdańsk, Poland*

Backgrounds

Bacterial biofilms are associated with a majority of chronic human infections. They are a serious challenge for today's medicine. Treatment of infections caused by biofilm-growing cells is linked to a high risk of failure due to an extreme resistance to antimicrobial agents and increased capacity to evade the host defenses. A large number of biofilm-associated infections involve *Staphylococcus aureus*. The biofilm forming *S. aureus* strains are responsible for causing a number of diseases, including infective endocarditis, ventilator associated pneumonia, otitis media and infections of bones. *S. aureus* infections are becoming more worrisome with the emergence of antibiotic resistant strains in particular methicillin-resistant *S. aureus* (MRSA).

Objectives

The aim of the study was the determination of biofilm forming ability of MRSA clinical isolates.

Methods

The total 70 isolates of MRSA were chosen from the collection of the Department of Medical Microbiology, Medical University of Gdańsk. The isolates were collected from different sites of infection, including wound, pus, furuncle, bronchial tree, throat and nose. Biofilms were formed *in vitro* by 48 h incubation of bacteria in 96 wells microtiter plates. Biofilm biomass was quantified using a crystal violet staining, while MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay assessed the number of viable cells.

Conclusions

All of the analyzed *S. aureus* isolates are able to form biofilms *in vitro*, but with different intensity. There was a correlation between the ability to form biofilm and the isolation site of *S. aureus*. Differences in a biofilm forming capacity may be caused by expression of various genes involved in biofilm formation.

FEMS7-1689
Pathogens / Pathogenicity - Part II

THE ANTITUBERCULOSIS DRUG BEDAQUILINE IS EFFICIENTLY NANOENCAPSULATED

A. Lucía[1,2,3], L. De Matteis[4], D. Jary[5,6], I. Serrano[7], S. García[4], F. Navarro[5,6], J. M. de la Fuente[7], J.A. Ainsa[1,2,3,8]
[1]Universidad de Zaragoza., Departamento de Microbiología, Zaragoza, Spain
[2]Instituto de Salud Carlos III, CIBER de Enfermedades Respiratorias CIBERES, Zaragoza, Spain
[3]Instituto de Investigación Sanitaria de Aragón, IIS-Aragón, Zaragoza, Spain
[4]Universidad de Zaragoza, Instituto de Nanociencia de Aragón INA, Zaragoza, Spain
[5]CEA- LETI- MINATEC Campus, Technologies for Healthcare and Biology division, Grenoble, France
[6]Univ. Grenoble, Alpes, Grenoble, France
[7]Universidad de Zaragoza-CSIC, Instituto de Ciencia de Materiales de Aragón ICMA, Zaragoza, Spain
[8]Universidad de Zaragoza, Instituto de Biocomputación y Física de Sistemas Complejos- BIFI, Zaragoza, Spain

Backgrounds

Antibiotic resistance in *Mycobacterium tuberculosis* is a global health emergency, and rising cases of MDR and XDR-TB are making increasingly difficult to treat tuberculosis. In 2012 the FDA approved the use of bedaquiline, the first drug designed to treat MDR-TB. It is very effective but shows serious side effects, so it can only be prescribed when no other options are available. Novel drug delivery systems based on nanocarriers are a promising strategy to overcome current therapeutic challenges. They improve drug solubility, protect the drugs, and allow a controlled release of the medication. Additionally they can be modified to allow selective transport to the sites of infection.

Objectives

The development of effective and safe nanotherapy is particularly relevant in the treatment of MDR-TB, as it requires very long treatments with highly toxic drugs. Therefore nanoencapsulation of bedaquiline is of special interest.

Methods

Chitosan based nanocapsules and Lipid NanoParticles, based on the Lipidots® technology, have been synthesized and optimized for the encapsulation of bedaquiline. upon quantification of drug loading efficiency, improvement of the drug payload, and nanoparticles stability amelioration in storage conditions. For the best candidates, drug release has been determined in biological media for in vitro assays. The antimycobacterial activity has been evaluated, and their cytotoxicity has been assayed in different cell lines.

Conclusions

Bedaquiline has been encapsulated in two different types of carriers, and the final nanoparticles have been totally characterized being found to be fully active as the free drug, and no cytotoxic.

FEMS7-0777
Pathogens / Pathogenicity - Part II

IN VITRO BIOFILM FORMATION BY STREPTOCOCCUS PNEUMONIAE WITH DIFFERENT SEROTYPES

M. Luo[1], N. Barua[1], M. IP[1]
[1]The Chinese University of Hong Kong, Microbiology, Shatin, Hong Kong S.A.R.

Backgrounds

The use of the pneumococcal conjugate vaccine has resulted in an increasing in non-vaccine serotypes colonizing the human nasopharynx. An increase of serotypes 15B/C, 15A/F, 23A, 3, 6C and 19A in Hong Kong has been observed since 2009. Biofilm formation occurs in nasopharynx colonization and enables pneumococci to evade the human immune responses and facilitate genetic exchanges to increase their fitness in the nasopharynx.

Objectives

The study aims to evaluate the biofilm-forming capacity of pneumococcal clinical isolates with different serotypes.

Methods

Biofilm of ATCC strain R6 and 47 clinical isolates with different serotypes were grown in 24-well polystyrene plates at 35˚C, 5% CO2 for 24 hours without shaking. Biofilms were then stained with 0.5% crystal violet and dissolved with 95% ethanol. The OD595 values were measured using spectrophotometer. All samples were run in triplicates.

Conclusions

Non-encapsulated strains formed the strongest biofilms among all the strains, following by serotype 14 strains, whereas serotype 3 strains formed the least biofilm. Serotype 6C and 6D strains showed high biofilm formation capacity, and were greater than serotype 6A or 6B strains. Biofilm biomass of the serotype 15A strain was much stronger than the 15B/C strains, while biofilms of the serotype 23A strains were slight less than those of serotype 23F strains. Serotype 19A strains formed more robust biofilms than 19F strains. Pneumococcal biofilm formation capacity varied among serotypes (mean OD value of non-encapsulated(n=3) >14(n=2) >15A(n=1) >19A(n=7) > 6C(n=2) >23F(n=2) >6D(n=2) >15B/C(n=2) >23A(n=2) > 6A(n=2) > 6B(n=2) >19F(16) >3(n=5)).

FEMS7-2574
Pathogens / Pathogenicity - Part II

RESISTANCE PROFILE, PRODUCTION OF EXOENZYMES AND HEMOLYSINS BY CLINICAL SAMPLES OF SERRATIA MARCESCENS

E. Lima Sousa[1], S. Alvares Martins[1], S.F. Rocha Constantino[1], W.D. Machado Maciel[1], I. Santos Sousa[2], N. Lisboa Camelo[2], S.C. Maia[2], F. Costa Moraes[3]
[1]Faculdade Estácio de São Luís, Biomedicina, São Luís, Brazil
[2]Faculdade Estácio de São Luís, Enfermagem, São Luís, Brazil
[3]Faculdade Estácio de São Luís, Enfermagem/Biomedicina, São Luís, Brazil

Backgrounds

The isolation of de *Serratia marcescens* in clinical samples of patients located in hospital reflects an unsatifying prognosis to these patients. Antimicrobial resistance and the production of diferente exoenzymes and hemolysins give this patogen virulence factors that lead to more severe affections of difficult treatment.

Objectives

To verify the resistance profile and the production of extracellular enzymes and hemolysins in clinical samples of *S. marcescens.*

Methods

21 strains of *S. marcescens* isolated from clinical samples from a laboratory in São Luís-MA were evaluated. The strains had their identification and resistance profile evaluated based on the automatized Vitek 2 method. The investigated exoenzymes were: amylase, phospholipase, protease and gelatinase. In order to do so, the Agar Muller Hinton was used with the addition of 1% of starch, 10% of egg yolk, 1% of olive oil, 10% of skim med milk and 4% of gelatine. The hemolisins were evidenced in Agar Blood 5% from lamb blood and human blood (A, B, O, AB). The formation of a light zone around the bacterial growth indicates positivity for hemolytic activity.

Conclusions

There was a predominance of strains from the tracheal secretion and all of them presented resistance to ampicilin, ampicilin sulbactam, cefuroxime and cefuroxime axetil. The strains didn't produce amylase or lipase. On the other hand, most of the strains presented gelatinase production capacity and only some of them were phospholips and protheolitical. According to the hemolytical pattern, the referred bacterial strains showed a stronger hemolytical capacity before human blood, specially type A group.

FEMS7-1968
Pathogens / Pathogenicity - Part II

BIOMASS AND METABOLIC ACTIVITIES OF CANDIDA PARAPSILOSIS COMPLEX BIOFILMS

N. Pena-Fernández[1], C. Marcos Arias[1], E. Mateo[1], E. Cantón[2], J. Pemán[2], E. Eraso[1], G. Quindós[1]
[1]Universidad del País Vasco UPV/EHU, Inmunología- Microbiología y Parasitología, Bilbao, Spain
[2]Hospital Universitari i Politècnic La Fe, Servicio de Microbiología, Valencia, Spain

Backgrounds

Candida parapsilosis complex is a common cause of catheter related bloodstream infection and a pathogen well known for its ability to survive on abiotic and biotic surfaces. However, little is known about the biofilm forming ability of the cryptic species of this complex.

Objectives

To evaluate biofilm production of *Candida parapsilosis* complex by measuring biomass and metabolic activities.

Methods

Biofilm production of 26 *Candida parapsilosis sensu stricto*, 11 *Candida orthopsilosis* and 3 *Candida metapsilosis* clinical isolates from Hospital Universitario y Politécnico La Fe (Valencia, Spain) was evaluated. Biofilms were developed on 100-well polystyrene microplates after 24 and 48 h incubation at 37 ºC. Afterwards, planktonic cells were removed for evaluating biofilm metabolic activity by a XTT colorimetric method and biomass production by crystal violet staining.

Conclusions

Candida parapsilosis biofilms showed significantly higher metabolic activity than *Candida orthopsilosis* ones (p=0.018 and p=0.048 at 24 and 48 h, respectively). Conversely, there were no differences with the metabolic activity of *Candida metapsilosis* biofilms. *Candida parapsilosis* showed more biofilm biomass production than *Candida orthopsilosis* and *Candida metapsilosis* (p=0.009 and p=0.0001 at 24 and 48 h, respectively). More biomass production and higher metabolic activities were observed at 48 h, although differences were not statistically significant.

Species of the *Candida parapsilosis* complex show ability to produce biofilms being *Candida parapsilosis sensu stricto* the most efficient biofilm developer.

Funding: Fundación ONCE and projects GIC15/78 IT990-16 (Gobierno Vasco/Eusko Jaurlaritza) and UFI 11/25 (UPV/EHU).

FEMS7-2960
Pathogens / Pathogenicity - Part II

EPIDEMICS OF MULTIDRUG-RESISTANT MYCOBACTERIUM TUBERCULOSIS HAARLEM GENOTYPE IN NORTHERN TUNISIA: A GENOTYPIC AND GENOMIC ANALYSIS

H. Mardassi[1]
[1]*Institut Pasteur de Tunis, Molecular Microbiology- vaccinology and Biotechnology development, TUNIS, Tunisia*

Backgrounds

Multidrug-resistant tuberculosis (MDR-TB) epidemics pose a great challenge for health-care authorities. Understanding how such rapidly transmitted forms of MDR-TB emerge is crucial to devise more appropriate intervention and surveillance strategies.

Objectives

Here we aim to explore thoroughly the transmission dynamics of the *Mycobacterium tuberculosis* Haarlem genotype in Northern Tunisia where it caused a major MDR-TB outbreak due to a Haarlem3-ST50 clone.

Methods

We ensured an 11-year (2001-2011) full coverage of the Haarlem genotype in the outbreak region by including all *M. tuberculosis* isolates displaying the Haarlem spoligotype signature (N = 253). We performed drug susceptibility testing and MIRU-VNTR24 (24-locus multiple interspersed repetitive unit-variable-number tandem repeat) typing for all isolates, as well as whole genome sequencing for 27 phylogenetically relevant isolates.

Conclusions

MIRU-VNTR24 typing revealed an exceptionally high clustering rate among both drug-susceptible and MDR-TB Haarlem isolates (78 % overall), suggesting rapid transmission. We identified a new MDR-TB Haarlem cluster, with no homolog (orphan) in the global spoligotype database, involving 7 patients. This minor MDR-TB cluster appears to have arisen from the same drug-susceptible progenitor as the major MDR-TB Haarlem clone (N = 48), a finding that was further confirmed by phylogenomics. Strikingly, in both MDR-TB clusters, pairwise genome comparisons uncovered much more genomic variation (14 to 83 single nucleotide polymorphism differences) than would usually be expected with a typical point source outbreak.

This study disclosed the ability of the Haarlem genotype to spread rapidly in Northern Tunisia, with evidence for an elevated mutation rate.

FEMS7-3128
Pathogens / Pathogenicity - Part II

TLR-2, SIGLEC-3 AND CD163 EXPRESSION ON PORCINE PERIPHERAL BLOOD MONOCYTES IS INCREASED DURING THE SEPSIS CAUSED BY HAEMOPHILUS PARASUIS

Á. Álvarez-Estrada[1], S. Martínez-Martínez[1], E.F. Rodríguez-Ferri[1], B. Álvarez-Vega[2], T. Fernández-Caballero[2], J. Domínguez-Juncal[2], C.B. Gutiérrez-Martín[1]
[1]University of León, Animal Health, León, Spain
[2]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA, Biotechnology Department, Madrid, Spain

Backgrounds

Scavenger receptor CD163 binds complexes between haptoglobin and damage-associated molecular patterns, and also acts in the recognition of intact bacteria. Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns. Most sialic acid-binding immunoglobulin-like lectins (Siglecs) recognize host sialic acid and act maintaining a balanced immune response. There is evidence that several bacterial pathogens, like genus *Haemophilus*, have developed different strategies for taking advantage of this interaction.

Objectives

To study the expression level changes of some of these molecules on peripheral blood (PB) monocytes from pigs suffering septicemia by *Haemophilus parasuis*.

Methods

Flow cytometry was used to evaluate TLR2, TLR4, Siglec-1, Siglec-3, Siglec-5 and CD163 expression level changes between PB monocytes from six pigs suffering experimental septicemia by *H. parasuis* and PB monocytes from the same pigs before infection.

Conclusions

Results showed a significant increase in the mean fluorescence intensity (ΔMFI) of TLR2 and Siglec-3 both on $CD163^+$ and $CD163^-$ monocytes during sepsis. A significant ΔMFI of CD163 during sepsis was also observed on these cells. No significant differences were seen for the remaining markers. We conclude that porcine monocytes show TLR-2, Siglec-3 and CD163 expression levels significantly higher during sepsis caused by *H. parasuis* than those of monocytes from healthy pigs. Further studies are required to know if these increases were due to changes in phenotype secondary to the interaction monocyte-bacteria or to release of monocytes with different maturation stages. Anyway, experimental infection by *H. parasuis* seems a good model to deepen in the knowledge of role of these molecules during sepsis.

FEMS7-1302
Pathogens / Pathogenicity - Part II

COMPARATIVE IN VIVO STUDY OF THE VIRULENCE PHENOTYPE BETWEEN DIFFERENT SPECIES FROM THE MYCOBACTERIUM TUBERCULOSIS COMPLEX

E. Mata[1], S. Uranga[1], C. Martín[1], N. Aguiló[1]
[1]Universidad de Zaragoza, Microbiología- Medicina Preventiva y Salud Pública, Zaragoza, Spain

Backgrounds

Bovine tuberculosis disease, caused by *Mycobacterium bovis*, represents a big problem for cattle industry in some countries in Europe and mainly in Africa. The understanding of its pathogenesis is crucial to develop an effective vaccine against this disease.

It has been observed that *M. bovis* is more virulent than *Mycobacterium tuberculosis* in several animal models (DunnPL.InfectImmun,1995, NedeltchevGG.InfectImmun,2009). Despite they share more than 99.95 % of genome homology (GarnierT.PNAS,2003) the genomic differences that drive to such a strong disparity remain unknown.

Objectives

Our target is to study the virulence of *M. bovis* vs *M. tuberculosis* and to explore the molecular basis responsible for the differences observed.

Methods

We have studied virulence and dissemination *in vivo* of two *M. tuberculosis* strains and the *M. bovis* reference strain AF2122 in the mouse model. Mice were inoculated intranasally and we evaluated histopathology of infected lungs and bacterial replication in lungs, spleen, liver and kidneys. We used GFP-expressing strains to study the infectivity and to characterize the cell populations infected through the fluorescence of lung cells.

We also study the expression *in vitro* of dormancy genes under stress culture conditions through qRT-PCR analysis.

Conclusions

We found that *M. bovis* strain has more ability to cause lung pathology and dissemination than *M. tuberculosis* strains, as well as higher capacity to infect host cells *in vivo.* A differential induction of the dormancy genes expression under stress conditions was found *in vitro* between *M. tuberculosis* and *M. bovis*, which may have an implication in the infection profile of these strains.

FEMS7-1416
Pathogens / Pathogenicity - Part II

LONG TERM INTRA-AMOEBAL SURVIVAL REDUCES VIRULENCE TRAITS OF PSEUDOMONAS AERUGINOSA

D. McDougald[1], C. Lutz[2], W. Leong[2], Y. Poh[2], B.Y.K. Yee[2], C.W. Qiang[2], M. Givskov[2]
[1]University of Technology Sydney, ithree Institute, Sydney, Australia
[2]Nanyang Technological University, Singapore Centre for Environmental Life Science Engineering, Singapore, Singapore

Backgrounds

The opportunistic pathogen, *Pseudomonas aeruginosa* is a major mortality factor for cystic fibrosis (CF) patients. Isolates of *P. aeruginosa* from CF patients show consistent genotypic and phenotypic changes compared to environmental and acute infection isolates, that are associated with the establishment of chronic infection. CF isolates often exhibit reduced virulence phenotypes, which are proposed to prevent host clearance, thus supporting their persistence in the lung.

Objectives

Here, we investigated the effects of long-term co-culture of *P. aeruginosa* with the amoeba *Acanthamoeba castellanii*.

Methods

Three independent co-cultures were established in low nutrient media, where naive *A. castellanii* were re-infected with amoeba-adapted *P. aeruginosa* populations every 3 days for 42 days. At days 3, 24 and 42, intracellular *P. aeruginosa* were isolated from *A. castellanii.* Control populations of *P. aeruginosa* that were not exposed to amoeba were also passaged for the same time period for comparison.

Conclusions

P. aeruginosa isolates exposed to *A. castellanii* for 42 days demonstrated decreased planktonic growth, reduced pyoverdine production and a reduction in twitching and swarming motilities. These differences were supported by genomic analysis that revealed single nucleotide polymorphisms in genes associated with pyoverdine synthesis and motility.

The phenotypic traits demonstrated by amoeba-adapted *P. aeruginosa* share similarities to *P. aeruginosa* isolates from CF lungs, suggesting that the selective factors experienced by the bacterium within amoeba are similar to those in the CF lung. Based on these data, we therefore suggest that the alteration of virulence may be a ubiquitous response of *P. aeruginosa* to survive the intra-host environment.

FEMS7-0405
Pathogens / Pathogenicity - Part II

GALLERIA MELLONELLA AS AN IN VIVO INFECTION MODEL FOR PATHOGENESIS, ANTIMICROBIAL DRUG AND DRUG-COMBINATION TESTING IN MYCOBACTERIUM ABSCESSUS

M. Meir[1], T. Grosfeld[2], B. Daniel[2]
[1]The Ruth Rappaport Children's Hospital- Rambam Health Care Campus, Pediatric Infectious Diseases Unit, Haifa, Israel
[2]The Hebrew University- Rehovot Campus, Koret School of Veterinary Medicine, Rehovot, Israel

Backgrounds

Treatment of *Mycobacterium abscessus* infections is extremely challenging due to its intrinsic resistance to most antibiotics. Research of virulence factors and antimicrobial treatments of *M. abscessus* is highly limited due to a lack of a practical *in vivo* model of infection. *Galleria mellonella* has been recently established as an infection model in various bacteria.

Objectives

To establish an easily feasible *in-vivo* model for *M. abscessus* infection, virulence, genetics and drug treatment in *G. mellonella* larvae.

Methods

We inoculated larvae with *M. abscessus*, then homogenized and cultured larvae at set time points. We also treated infected larvae with antibiotics and examined the effect on CFU counts.

Conclusions

Larvae were inoculated with *M. abscessus* by injection on day 0. We injected meropenem, tigecycline or both to larvae in treatment groups on days 2 and 3. We demonstrated a consistent rise in mycobacterial counts on days 1-5 of infection, and a consistent time to mortality curve in untreated controls. On day 7, meropenem treated larvae had lower CFU counts by 1-2 logs compared to untreated controls. Mortality in the meropenem group was postponed by 48 hours compared to controls. The effect of tigecycline was neither additive nor synergistic to the effect of meropenem. These findings suggest *G. mellonella* to be an adequate *in vivo* model of *M. abscessus* infection that may be used for efficacy assessment of various antimicrobials and antimicrobial combination. The *G. mellonella* model may also be used for future studies of pathogenesis and virulence factors of *M. abscesssus*.

FEMS7-0818
Pathogens / Pathogenicity - Part II

GENOMIC ANALYSIS OF DIFFUSELY ADHERENT ESCHERICHIA COLI AND ENTEROAGGREGATIVE ESCHERICHIA COLI STRAINS ISOLATED FROM PATIENTS WITH MODERATE TO SEVERE DIARRHOEA

M. Meza-Segura[1], A. Vera-Ponce de Leon[2], E. Martinez-Romero[2], M. Zaidi[3,4], J. Nataro[5], T. Estrada-Garcia[1]
[1]CINVESTAV, Molecular Biomedicine, Mexico City, Mexico
[2]UNAM, Center for Genomic Sciences, Cuernavaca, Mexico
[3]Hospital General O'Horan, Infectious Diseases Research Unit, Merida, Mexico
[4]Michigan State University, Department of Epidemiology and Biostatistics, Lansing, USA
[5]University of Virginia, Pediatrics, Charlottesville, USA

Backgrounds

Diarrhoea remains a major cause of morbidity and mortality among young children <5 years old in less developed countries. Diffusely adherent *Escherichia coli* (DAEC) and enteroaggregative *E. coli* (EAEC) are the main agents associated with acute diarrhoea in children requiring hospitalisation, in Mexico, surpassing *Salmonella* and *Shigella*.

Objectives

To establish the prevalence of virulence factor genes (VFG) in DAEC and EAEC genomes from cases of moderate to severe diarrhoea and to analyse the phylogenetic relationships between DAEC and EAEC isolates with other pathogenic (diarrheagenic and extraintestinal) and commensal *E. coli*.

Methods

Genomes of ten DAEC and nine EAEC isolated from young children and one EAEC from an adult with severe or moderate diarrhoea were sequenced using Illumina MiSeq. Genomes were analysed for the presence of VFG (encoding toxins, proteases, invasins, adhesins, flagella, type secretion systems (TSS), complement-resistance-factors, and iron acquisition systems) by BLAST. Pan-genome and core-genome phylogenetic trees were built including 86 previously reported genomes, using get-homologues and PhyML, respectively.

Conclusions

Significantly more VFG were identified among DAEC and EAEC genomes than in commensal strains ($p<0.05$). Toxins, proteases, and the Aai T6SS were the most prevalent VFG among EAEC strains, while in DAEC iron acquisition systems and complement resistance factor genes. Also, were identified two new DAEC Afa/Dr/Daa adhesin operons and the presence of the enterotoxigenic *E. coli* CS22 fimbria in two EAEC strains. EAEC and DAEC strains clustered in four and three phylogenetic groups, respectively, and three of these groups contained both DAEC and EAEC strains.

FEMS7-1247
Pathogens / Pathogenicity - Part II

DETECTION OF H. PYLORI MICROEVOLUTION AND MULTIPLE INFECTION FROM GASTRIC BIOPSIES BY AMPLICON SEQUENCING

M. Palau[1], A.M. Comeau[2], N. Piqué[1], M.J. Ramírez-Lázaro[3], S. Lario[3], X. Calvet[3], M.G.I. Langille[2], D. Miñana-Galbis[1]
[1]Universitat de Barcelona- Facultat de Farmàcia i Ciències de l'Alimentació, Biologia- Sanitat i Medi Ambient- Secció de Microbiologia, Barcelona- Catalonia, Spain
[2]CGEB-Integrated Microbiome Resource IMR, Pharmacology- Dalhousie University, Halifax, Canada
[3]Institut Universitari Parc Taulí-UAB, Digestive Diseases Service- Hospital de Sabadell- Corporació Sanitària Parc Taulí, Sabadell- Catalonia, Spain

Backgrounds

Despite the extensive literature published on *H. pylori*, the exact mode of transmission (oral-oral, gastro-oral, and fecal-oral) and the role of *H. pylori* multiple infection are still controversial.

Objectives

The aim of this study was to evaluate the usefulness of amplicon sequencing methodology for the detection of *H. pylori* microevolution and multiple infection from gastric biopsies of patients with dyspeptic symptoms (from atrophy to gastric cancer).

Methods

DNA was extracted from five gastric biopsies (B508S and B508T belonging to the same patient – B508S from normal tissue and B508T from gastric adenocarcinoma–, B247S from normal tissue of a patient with gastric cancer, B373 with metaplasia, and B601A with atrophy). Housekeeping genes *luxS* (autoinducer-2 synthase) and *cgt* (cholesterol-α-glucosyltransferase) were amplified and sequenced following manufacturer instructions using the GS Junior system (Roche). Sequences were analysed with Mothur, aligned with Mega6, edited in BioEdit, and clustered at 97% identity.

Conclusions

The *luxS* sequences clustered into 12 unique OTUs (operational taxonomic units), with a range of 2–5 OTUs being present per gastric biopsy, and four OTUs representing 91% of all sequences and belonging to the samples B508S and B508T, B247S, B373 and B601A, respectively. Analysis of *cgt* sequences is in process.
Results suggest that gastric mucosa is infected with multiple *H. pylori* strains, where frequent microevolution events from a predominant *H. pylori* strain coexisting with other minority strains.

FEMS7-1015
Pathogens / Pathogenicity - Part II

SERUM ALBUMIN BINDING OF BDELLOVIBRIO BACTERIOVORUS: INHIBITION OF PREDATORY ACTIVITY AGAINST HUMAN PATHOGENS

R. Mitchell[1], *C.M. Ghim*[1]
[1]*Ulsan National Institute of Science and Technology UNIST, School of Life Sciences, Ulsan, Republic of Korea*

Backgrounds

Bdellovibrio bacteriovorus is a bacterial predator that actively attacks and consumes other Gram-negative bacteria. The list of prey includes many human pathogens, including strains of *Salmonella*, *Escherichia*, *Yersinia* and *Acinetobacter*. As bacteria can also cause sepsis, we undertook this study to evaluate the ability of *B. bacteriovorus* to predate on *E. coli*, *S. enterica* and *Klebsiella pneumoniae* within human serum.

Objectives

This study sought to identify the factors present in human serum and their impact on the activity of *B. bacteriovorus*, particularly against human pathogens. These included the serum complement activity against each of the bacterial strains tested, the impact of the serum osmolality and the effect serum albumin had on the predatory activity against each of the prey strains.

Methods

The activity of the serum complement and predator were both evaluated by determining the bacterial viabilities under each condition. The osmolality tests were performed using various saline solutions and measuring the ability of the predator to attack *E. coli*. The serum albumin tests include using a bioluminescent prey as well as microscopic imaging techniques.

Conclusions

While serum complement was active against *E. coli*, *S. enterica* and *K. pneumoniae*, it was not against the predator, *B. bacteriovorus*. However, both the serum osmolality and albumin inhibited predation. The osmolality of serum was 268 mOsm/kg, a level that blocked the activity of *B. bacteriovorus*. As well, serum albumin coated the predator, effectively inhibiting it from attacking its prey. Consequently, predation does not seem to be a viable option to treat pathogens within human serum.

FEMS7-1035
Pathogens / Pathogenicity - Part II

BDELLOVIBRIO BACTERIOVORUS: EFFECTIVE AGAINST BACTERIAL BIOFILMS, GENTLE TOWARDS HUMAN CELLS

R. Mitchell[1]
[1]*Ulsan National Institute of Science and Technology UNIST, School of Life Sciences, Ulsan, Republic of Korea*

Backgrounds

Bacteria in nature, including in and on the human body, are often found in biofilms, communities of cells connected to each other and a surface. Not only are biofilms naturally resilient, they also increase the antibiotic resistance of the members present within them. Consequently, a means to remove biofilms that is effective, yet gentle enough to use, is needed.

Objectives

Bacterial predators, including *Bdellovibio bacteriovorus*, are capable of attacking and consuming other Gram-negative bacteria, including numerous human pathogens. They have also be shown to effectively attack biofilms of these species, reducing their populations by several log. Here we sought to evaluate the activity of *B. bacteriovorus* against non-prey and polymicrobial biofilms, as well as in the removal of biofilms from the surface of human cells.

Methods

Biofilms were formed either on abiotic of biotic surfaces. For polymicrobial studies, the bacterial strains were mixed prior to incubation to generate the biofilms. We used various microscopic techniques to evaluate biofilm responses to predation, as well as the human response to the predatory bacteria. The cytokine responses were measured using ELISA.

Conclusions

Our results show that predatory bacteria can remove both single and polymicrobial biofilms from the surface through predation and secretion of proteases. However, they were not harmful to human cells, eliciting no or only weak cytokine responses but no cell death. Biofilms formed on human cells were also effectively removed by *B. bacteriovorus*, while the underlying MCF-10a cells were protected, showing its gentle nature towards human cells but tough character against bacterial biofilms.

FEMS7-1749
Pathogens / Pathogenicity - Part II

EVALUATING THE ANTIMICROBIAL EFFECT OF THE BACTERIOCIN AS-48 ALONE AND IN COMBINATION WITH LYSOZYME AGAINST PROPIONIBACTERIUM ACNES

R. Cebrián[1], S. Arévalo[1], S. Ananou[1], M. Montalbán López[1], S. Arias-Santiago[2], C. Riazzo[3], M.D. Rojo[3], P. Bermúdez[4], E. Valdivia[1], M. Martínez-Bueno[1], M. Maqueda[1]
[1]University of Granada, Microbiology, Granada, Spain
[2]Hospital Universitario Virgen de las Nieves, Dermatology, Granada, Spain
[3]Hospital Universitario Virgen de las Nieves, Microbiology, Granada, Spain
[4]Hospital Regional Universitario, Microbiology, Malaga, Spain

Backgrounds

Propionibacterium acnes is involved in acne vulgaris and can be related to other infectious diseases. In the last years the multidrug resistant (MDR) isolates of this bacterium have increased alarmingly. AS-48 is a potent head-to-tail cyclized bacteriocin produced by some Enterococci.

Objectives

Our goal is to assess the activity of AS-48 against *P. acnes*.

Methods

We have evaluated the minimal inhibitory concentration of AS-48 and combinations with other antimicrobials on 23 different clinical isolates (from skin, bone, abscess or blood), some of which were resistant to erythromycin and clindamycin, the most commonly used antibiotics in the treatment of acne.

Conclusions

Our results show that AS-48 can effectively inhibit the clinical strains tested, including the MDR strains, at concentrations ranging between 2-12 µg/mL. We prove a bactericidal effect at concentrations above 1 µg/mL for one of the strains. Moreover, the combinations of AS-48 with either palmitic acid or lysozyme displayed a significant synergistic effect. The combined effect with lysozyme rendered the best reduction in MIC values (4-10 fold reduction against MDR strains). This combination was formulated in a cream and tested in a swine ear infection model causing a 4-log reduction of *P. acnes* in 1 h.

Our data prove the effectiveness of the enterocin AS-48 alone and in combination with lysozyme for the control of (MDR) *P. acnes* and highlight its potential in skin formulations, being particularly promising in light of emerging antibiotic resistance. The results confirm that this composition could be considered an approach for medical and pharmaceutical purposes against *P. acnes*.

Acknowledgements

This research was funded by SAF2013-48971-C2-1-R with funds from ERDF and BIO160 (UGR).

FEMS7-2967
Pathogens / Pathogenicity - Part II

EVALUATION OF THE ENTEROCIN AS-48 FOR THE CONTROL OF GARDNERELLA VAGINALIS

S. Arevalo[1], R. Cebrián[1], M. Montalbán López[1], J. Liebana Ureña[1], M. Martinez-Bueno[1], M. Maqueda[1], E. Valdivia[1]
[1]University of Granada, Microbiology, Granada, Spain

Backgrounds

Bacterial vaginosis (BV) is a common infectious disease characterized by an imbalance in the vaginal microbiota, where healthy lactobacilli are replaced by a proliferation of (facultative) anaerobic microorganisms, notably *Gardnerella vaginalis*. Antibiotic treatment of BV is not always effective and often leads to reoccurrence of the infection due, in many cases, to the arising of antimicrobial resistance.

The enterocin AS-48 is a circular cationic antimicrobial peptide produced by diverse *Enterococcus* species that selectively induces pore formation in bacterial membranes.

Objectives

Our research objectives were to reliably identify *G. vaginalis* using molecular techniques and to assay the inhibitory activity of the enterocin AS-48.

Methods

We employed PCR with designed specific primers (VG10F/534R) derived from the 16S rDNA of *G. vaginalis* for a quick identification of new isolates. The antimicrobial activity of AS-48 against the isolates was assessed by spot-on-lawn onto agar *Gardnerella* and by transmission electron microscopy.

Conclusions

We established that specific PCR is a reliable identification technique. We isolated 29 strains and 28 of them were sensitive to less than 75 ng of AS-48. TEM images of *G. vaginalis* treated with AS-48 (60 µg/ml for 1h) revealed the absence of lysis. Although the treated cells maintained the integrity of the cell wall, they presented evident citoplasmatic membrane retractions, due possibly to the loss of intracellular content.

These results reinforce the continuation of the studies on AS-48 for the control of *G. vaginalis*.

Acknowledgements. This research was funded by SAF2013-48971-C2-1-R with funds from ERDF and BIO160 (UGR).

FEMS7-0760
Pathogens / Pathogenicity - Part II

SEAGULLS, BIRDS OF PREY AND FOXES: IMPORTANT CARRIERS OF ESBL-PRODUCING ESCHERICHIA COLI IN THE NORTH-WEST OF SPAIN AND NOW PRESENCE OF COLISTIN-RESISTANCE

A. Mora Gutiérrez[1], S. Viso González[1], A.M. López Beceiro[2], L.F. Fidalgo Álvarez[2], M. Blanco Álvarez[1]
[1]Universidad de Santiago de Compostela, Microbioloxía e Parasitoloxía, Lugo, Spain
[2]Universidad de Santiago de Compostela, Anatomía- Producción Animal y Ciencias Clínicas Veterinarias, Lugo, Spain

Backgrounds

E. coli strains producing extended-spectrum beta-lactamases (ESBL-*E. coli*) and other beta-lactamases represent a major problem in both human and veterinary medicine. Wild animals can become colonized while sharing the same habitat as domestic animals and humans or through environmental pollution.

Objectives

The objective of this study was to define the role of wildlife populations that have undergone a significant increase over the last decades in the north-west of Spain as reservoir of ESBL-E. coli strains.

Methods

The faecal samples of 620 wild animals (390 birds and 230 mammals) obtained during 2012-2015 and belonging to 7 different groups were examined by PCR for the presence of ESBL and pAmpC-producing *E. coli*. As a result, 13,4% (83 of 620) were ESBL-carriers with isolation from the 7 animal groups analyzed. Seagulls (23,4%), foxes (16,3%) and birds of prey (15,5%) showed the highest significant prevalences. The 95 isolates recovered were typed as SHV-12 (29,5%), CTX-M-1 (26,3%), CTX-M-14 (18,9%), CTX-M-32 (12,6%), CTX-M-15 (5,3%), CTX-M-9 (4,2%), CTX-M-27 (2,1%), CTX-M-24 (1,0%), with 2 isolates being additionally CIT.

Furthermore, 1 strain isolated from a fox was positive for *mcr*-1. By conventional molecular characterization was O150:H20-A-ST1324 CTX-M-1. By whole genome sequencing, the mcr-1 was located on an IncX4 plasmid, and several additional resistant genes were detected (*floR*, *dfrA12*, *drfA17*, *sul1/2/3*, *cmlA1*, *mef(B)*, *aadA2*, *aadA5*, *tet(A)*, *tet(M)*, *aadA1*, $bla_{\text{TEM-1}}$).**Conclusions**

This is the first report of a plasmid-mediated colistin-resistant ESBL-*E. coli* in fox. We suggest seagulls, foxes and birds of prey as possible sentinels in surveillance programs on ESBL dissemination.

FEMS7-2349
Pathogens / Pathogenicity - Part II

DIFFERENTIAL EFFECT OF THE AGGREGATIVE ADHERENCE FIMBRIA TYPE II ON THE INTESTINAL TRACT OF MICE INFECTED WITH ENTEROAGGREGATIVE ESCHERICHIA COLI WITH AN UNDISTURBED MICROBIOTA

N. Moran-Garcia[1], L.S. Catalina[1], G.G. Silvia[2], T. Victor[2], F.L. Alfonso[1], S. Michael[1], N. James P.[3], E.G. Teresa[1]
[1]CINVESTAV, Molecular Biomedicine, Cd. de México, Mexico
[2]CINVESTAV, Infectomic and Pathogenesis, Cd. de México, Mexico
[3]University of Virginia School of Medicine, Pediatrics, Charlottesville, USA

Backgrounds

Enteroaggregative *Escherichia coli* (EAEC) is one of the major agents causing diarrhoea in young children from developing countries and adults from industrialised regions. EAEC adherence fimbria (AAF) are responsible for the EAEC characteristic aggregative phenotype.

Objectives

To evaluate *in situ* the role of AAF/II fimbria using a recently developed mouse model of EAEC infection with an undisturbed microbiota.

Methods

Six week old, C57BL/6 mice, without previous antibiotic treatment, were orally inoculated with $5x10^9$ CFU of 042 EAEC archetype strain that express the AAF/II fimbria, while another set of mice were infected with the same dose of an 042 AFF/II mutant strain. Control mice were only given saline. Colon and Ileum paraffin sections were analyzed for morphological changes by H&E staining and for β-catenin and Muc1 expression by immunofluorescence.

Conclusions

On H&E stain, no apparent histopathologic changes were observed in the intestines of animals infected with 042 or ΔAAF/II-042. However, in 042-infected mice, β-catenin was partially delocalized from the enterocyte basolateral location in ileal sections, and was apparently more abundant in the cytoplasm, appearing along the apical enterocyte surface at both days 3 and 4 post-inoculation; concurrently, the Muc1 was highly expressed on ileal enterocytes at 3 days post-infection. In ΔAAF/II-042 infected mice, β-catenin was not delocalized, nor was Muc1 expressed. Noteworthy, Muc1 was not expressed nor was β-catenin delocalized to the cytoplasm in colonic enterocytes of mice infected with either strain. EAEC AAF/II fimbria plays a role in β-catenin delocalization and expression of Muc1 in ileal enterocytes of 042 infected mice.

FEMS7-0450
Pathogens / Pathogenicity - Part II

SIZE REALLY MATTERS: O-ANTIGEN FINAL LENGTH HAS AN ESSENTIAL ROLE DURING MICROBIAL PATHOGENESIS

C. Moreira[1]
[1]*School of Phamaceutical Sciences, Biological Sciences, Araraquara, Brazil*

Backgrounds

Salmonella Typhimurium is a major foodborne pathogen responsible for gastroenteritis. The LPS is employed to protect themselves against host defenses, more specifically the O-antigen (OAg) portion plays an essential role during the infection and as immunogenic defensive shield. The O-Ag assembly in Salmonella Typhimurium has the Wzz system to determine the O-antigen final chain length and it presents a tri-modal distribution. Therefore the chain length may be Short (16units), Long (35units), and Very-Long O-Ag forms (more than 100 unit repeats). The WzzST and WzzfepE proteins regulate respective the L O-Ag and VL O-Ag synthesis. Recently we have reported the role of VisP (Virulence and stress related Periplasmic protein) and LpxO enzyme in chemical signaling during pathogenesis (Moreira et al, 2013). Whereas the VisP prompts Salmonella Typhimurium to survive upon stress factors, and LpxO-mediated Lipid A hydroxylation helps the bacteria to adapt itself to host defenses and different colonization niches.

Objectives

A better understanding of the Salmonella Typhimurium chemical signaling during pathogenesis. Moreover, elucidate how the O-antigen, flagella, T3SS, and the periplasmic VisP protein converge to help bacteria to survive and infect their hosts.

Methods

We have assessed epithelial cell invasion, J774 macrophages intracellular replication, and flagella motility differences employed the λ Red single and double-mutants of the wzzST, wzzfepE, visP, and lpxO genes to compared with Salmonella Typhimurium WT levels. The VL-OAg has shown to be detrimental to invasion, intracellular replication and flagella motility, independent of the L-OAg form presence. The VL-OAg diminished flagella motility by 65%, decreased cells invasion, and slightly reduced intracellular survival compared to the WT levels.

Conclusions

How bacteria adapt themselves to these changes and regulate their membrane to interact with the host are essential aspects to microbial-host relationships. The complete elucidation of all these mechanisms will be essential to understand their relationship, develop novel technologies and therapies

FEMS7-3081
Pathogens / Pathogenicity - Part II

18S RRNA AMPLICON-BASED METAGENOMICS FOR MULTIPLE IDENTIFICATION OF WATERBORNE PROTOZOA IN IRRIGATION WATER

Y. Moreno[1], R. Pérez[1], L. Moreno-Mesonero[1], I. Amoros[1], J.L. Alonso[1]
[1]University Research Institute of Water and Environmental Engineering. UPV, Water Chemistry and Microbiology division, VAlencia, Spain

Backgrounds

The contamination of drinking and bathing water with protozoan pathogens and the usage of sewage water for agricultural purposes poses a serious threat to millions of people worldwide. Among the waterborne pathogens, *Giardia* and *Cryptosporidium* are the most common causes of major diarrheal outbreaks globally. In contrast, insufficient information is available for *Toxoplasma gondii*, *Blastocystis hominis*, , *Entamoeba histolytica* and other free-living amoebae.

Molecular techniques are the most promising methods in comparison with conventional methods, which much benefit the water industry and public health. 18S rRNA amplicon-based next generation sequencing could be a powerful tool to investigate the presence of protozoan pathogens in the environment**Objectives**

The aim of this study has been to use 18S rRNA amplicon- sequencing to identify simultaneously the presence of important waterborne protozoa in irrigation water.

Methods

Five irrigation water samples were collected. DNA was extracted using UNEX protocol (homogenization using the FastPrep-24® Instrument at speed setting 6.5,120 s). PCR was performed as described by Illumina guide. A pair of primers were designed to target the V4 region of the18S rRNA gene of the protozoa. DNA sequencing data was processed using QIIME™ 1.8.0 open – source bioinformatics pipeline (http://qiime.org).

Conclusions

Pathogenic protozoa identified by 18S rRNA sequencing included *Blastocystis* (32 OTUs) and *Entamoeba* (38 OTUs). Other identified genus, such as *Giardia* (12 OTUs), *Acanthamoeba* (3 OTUs) and *Toxoplasma* (5 OTUs), had a lower representation.

Then, this technique could be established as a useful and cost-effective method to identify simultaneously, within a single analysis important pathogenic protozoa in water samples.

FEMS7-2893
Pathogens / Pathogenicity - Part II

GENETIC DISSECTION OF THE SIGNALING CASCADE THAT CONTROLS ACTIVATION OF THE SHIGELLA TYPE III SECRETION SYSTEM FROM THE NEEDLE TIP

I. Murillo[1], I. Martinez Argudo[2], A. Blocker[1]
[1]University of Bristol, Cellular and Molecular Medicine, BRISTOL, United Kingdom
[2]Universitdad de Castilla-La Mancha-, Área de Genética, Toledo, Spain

Backgrounds

Many Gram-negative bacterial pathogens use type III secretion systems (T3SSs) for virulence. The *Shigella* T3SS consists of a hollow needle, made of MxiH and protruding from the bacterial surface, anchored in both bacterial membranes by multimeric protein rings. Atop the needle lies the tip complex (TC), formed by IpaD and IpaB. Upon physical contact with eukaryotic host cells, T3S is initiated leading to formation of a pore in the eukaryotic cell membrane, which is made of IpaB and IpaC. Through the needle and pore channels, further bacterial proteins are translocated inside the host cell to meditate its invasion. IpaD and the needle are implicated in transduction of the host cell-sensing signal to the T3S apparatus. Furthermore, the sensing-competent TC seems formed of 4 IpaDs topped by 1 IpaB. However, nothing further is known about the activation process.

Objectives

To investigate IpaB's role during T3SS activation and whether IpaB is directly involved in host sensing by isolating and characterising *ipaB* mutants unresponsive to activation signals.

Methods

Isolation of secretion-deregulated IpaB mutants using random mutagenesis and a genetic screen.

Conclusions

We found *ipaB* point mutations in leading to defects in secretion activation, which sometimes diminished pore insertion and host cell invasion. We also demonstrated IpaB communicates intramolecularly and intermolecularly with IpaD and MxiH within the TC because mutations affecting these interactions impair signal transduction.

FEMS7-1876
Pathogens / Pathogenicity - Part II

PATHOGENICITY GENES FROM PLASMIDS OF PSEUDOMONAS SYRINGAE PV. SAVASTANOI ARE MAINTAINED IN THE POPULATION BY DIFFERENT EVOLUTIONARY FORCES

M. Añorga[1], A. Pintado[2], L. Bardaji[1], C.J. Ramos[2], J. Murillo[1]
[1]Universidad Pública de Navarra, Departamento de Producción Agraria, Pamplona, Spain
[2]Instituto de Hortofruticultura Subtropical y Mediterránea «La Mayora»- Universidad de Málaga-CSIC, Área de Genética, Málaga, Spain

Backgrounds

Pseudomonas syringae pv. savastanoi NCPPB3335 is a plant pathogen that naturally causes aerial tumours on olive (*Olea europaea*). Pathogenicity is dependent on several determinants, including a functional type III secretion system and the production of phytohormones.

Objectives

We sought to investigate the role of native plasmids in pathogenicity and virulence, as well as the mechanisms involved in their genetic and structural stability.

Methods

By functional inactivation of stability determinants and spontaneous curing, we obtained a derivative of strain NCPPB3335 cured of its three native plasmids (strain UPN912) and unable to induce disease symptoms. Pathogenicity and virulence were tested in micropropagated and lignified olive plants cv. Arbequina.

Conclusions

Genes *ptz* and *ipt* are both essential for the production of tumours in plantlets, and probably involved in the biosynthesis of cytokinins. Gene *ptz* codes for an isopentenyl transferase and is located in plasmid pPsv48A whereas *ipt* codes for a putative isopentenyl diphosphate isomerase and is located in pPsv48C. Insertion sequence IS*801* promoted the occurrence of deletions (average frequency of $1.8\pm0.7 \times 10^{-4}$) by one-ended transposition in plasmid pPsv48C, of which half resulted in the loss of gene *ipt*. Functional inactivation of the three toxin–antitoxin systems from pPsv48C increased three times the frequency of deletions, with 80% of them losing gene *ipt*. Together, our results indicate that maintenance of pathogenicity genes in bacterial populations of *P. syringae*, and their allocation to plasmids, results from the combination of diverse antagonistic evolutionary forces that are unrelated and independent of the pathogenicity process.

FEMS7-1146
Pathogens / Pathogenicity - Part II

DECIPHERING THE TRANSCRIPTIONAL REPROGRAMMING OF YERSINIA FOR PERSISTENCE

R. Navais[1], *M. Fällman*[1]
[1]*Umeå University, Molecular Biology, Umeå, Sweden*

Backgrounds

Yersinia pseudotuberculosis is an enteropathogen causing gastroenteritis, which usually is self-limiting in humans. This enteropathogen provides a suitable mouse model system for persistent bacterial infections. It invades and persist in extracellular foci in lymphoid follicles in cecum where it is surrounded by polymorphonuclear leukocytes. An *in vivo* RNA-seq approach was recently used to identify the genes whose expression is increased during *Y. pseudotuberculosis* persistence. This approach revealed that *Y. pseudotuberculosis* reprograms it transcriptome for persistence, involving repression of T3SS and induction of flagella and genes encoding proteins involved in anaerobiosis, chemotaxis and protection against oxidative and acidic stress, indicating the influence of and adaptation to different environmental cues. All this suggests a model for the life cycle of this enteropathogen with reprogramming from a virulent phenotype during early infection to an adapted phenotype during late infection or persistence. The observed transcriptional reprogramming has become a major focus of attention in our research group.

Objectives

We aim to identify the genes that control this novel regulation as well as the environmental cues that induce persistence.

Methods

Reporter strains harbouring a transcriptional fusion between the selected promoter (up- or down-regulated during persistence) and a chloramphenicol resistance gene have been generated. These reporter strains have been subjected to transposon mutagenesis and the resulting transposon mutant libraries have been screened for the phenotype of interest.

Conclusions

Two promoters that are downregulated during persistence seem to be calcium-sensitive. Further studies are needed to assess whether calcium plays a role in triggering the transcriptional reprogramming of *Yersinia* for persistence.

FEMS7-0016
Pathogens / Pathogenicity - Part II

BIOLOGICAL FEATURE OF E. COLI STRAINS ISOLATED FROM PIGLETS WITH ENTERITIS

O. Nechypurenko[1], D. Dreval[2], T. Ponomarenko[3], I. Sobko[4], A. Pastyria[5]
[1]D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine, Antibiotics department, Kyiv, Ukraine
[2]Centre of Veterinary Diagnostics, Bacteriology, Kyiv, Ukraine
[3]Centre of Veterinary Diagnostics, Molecular diagnostics, Kyiv, Ukraine
[4]Centre of Veterinary Diagnostics, Director, Kyiv, Ukraine
[5]Centre of Veterinary Diagnostics, Research, Kyiv, Ukraine

Backgrounds

Escherichiosis is a currently distributed worldwide disease. *E. coli* is a representative of opportunistic microbiota of gastrointestinal tract of humans and animals, but pathogenic strains have virulence factors that induce the development of enteritis, edema disease and septicemia. Such bacterial isolates are the main cause of intestinal disorders in porcine farming of Ukraine. Besides the pathogenic *E. coli* strains could affect human, be the source of food poisoning and transmission of antibiotic resistance to clinical isolates.

Objectives

Investigate the biological features of *E. coli* strains isolated from piglets with enteritis and determine the dynamics of their antibiotic resistance development through 2011-2015 years in Ukraine.

Methods

Routine autopsy of piglets with intestinal disorders at the age up to 60 days, isolation of bacteria from the small intestine and identification of isolates using Api 20E test, PCR detection of pathogenic strains, disk-diffusion determination of antibiotic resistance (13 antimicrobials), standard histological examination of affected organs.

Conclusions

During 2011-2015 years we analyzed 357 piglets from 135 farms of Ukraine. From the small intestine of 311 animals 336 strains were isolated among which 317 were identified as pathogenic *E. coli*. The amount of 66 enteropathogenic, 233 enterotoxigenic, and 18 enterohemorrhagic bacterial strains have been differentiated based on histopathology and PCR detection of STa, STb, LT, Stx toxins and intimin genes. It was determined that colistin, gentamicine, enrofloxacine and florfenicol were the most effective antibiotics because of 71%, 66%, 53% and 55% of isolated *E.coli* strains were susceptible to these medications respectively. However 5 strains of *E.coli* were resistant to all of used antimicrobials. Also in 2015 the increasement of number of resistant strains was found to colistin, enrofloxacine and florfenicol in 1.5-2.0 times comparatively to 2011 year. Therefore it was determined the distribution of pathogenic *E.coli* strains and the dynamics of their antibiotic resistance development in Ukraine.

FEMS7-0390
Pathogens / Pathogenicity - Part II

MOLECULAR CHARACTERIZATION OF PSEUDOMONAS SYRINGAE STRAINS FROM SERBIA BY USING MULTI-LOCUS SEQUENCE TYPING METHOD

I. Nikolić[1], T. Popović[2], T. Berić[1], J. Lozo[3], I. Dimkić[1], D. Fira[3], S. Stanković[1]
[1]Faculty of Biology - University of Belgrade, Department of Microbiology, Belgrade, Serbia
[2]Institute for Plant Protection and Environment, Department of Plant Disease, Belgrade, Serbia
[3]Faculty of Biology - University of Belgrade, Department of Biochemistry and Molecular Biology, Belgrade, Serbia

Backgrounds

Pseudomonas syringae represent a phylogenetic complex of plant pathogenic strains, which affect different host plants such as fruits trees, field crops, vegetables and ornamental plants. During 2013, leaf spot disease of sugar beet caused by *P. syringae* was detected for a first time in Serbia.

Objectives

Objectives of our research were to characterize *P. syringae* strains from Serbia by using multi-locus sequence typing (MLST) method, in order to estimate intra-species genetic diversity.

Methods

Twenty five strains of *Pseudomonas syringae,* out of 104 identified, from fourteen commercial fields in province Vojvodina in Serbia were tested. Multi-locus sequence typing was performed with strains of *P. syringae* from Serbia and the three reference strains on four housekeeping genes (*gyrB*, *rpoD*, *gapA*, *gltA*). Sequences were analyzed in MEGA 6 software package and phylogenetic tree was constructed by using Neighbor-Joining clustering method.

Conclusions

Genetic differences among tested strains of *P. syringae* obtained by MLST method, show high level of genetic diversity by analyzing *gyrB* and *gapA* genes, which includes existence of four and three genetic groups, respectively. In contrast, genes *rpoD* and *gltA* did not show genetic variations among tested strains. Obtained genetic diversity demonstrates that infection of sugar beet with *P. syringae* in Vojvodina was not clonal and there were at least three different lines of infection. According to obtained results, analysis of *gyrB* and *gapA* genes was shown as reliable and suitable for determination of genetic diversity of *P. syringae*.

FEMS7-2063
Pathogens / Pathogenicity - Part II

FIRST DESCRIPTION OF METHICILLIN-LINEZOLID RESISTANT STAPHYLOCOCCUS EPIDERMIDIS WITHOUT PREVIOUS LINEZOLID EXPOSURE, TURKISH EAST BLACKSEA REGION 2016

A.R. Freitas[1], A.R. Dilek[2], L. Peixe[1], C. Novais[1]
[1]REQUIMTE/UCIBIO- Faculty of Pharmacy- University of Porto, Microbiology, Porto, Portugal
[2]Recep Tayyip Erdoğan University. Medical Faculty Hospital, Microbiology, Rize, Turkey

Backgrounds

Linezolid is a critical option for multidrug-resistant Gram-positive infections. Emergence of resistant strains is occasionally described, mostly associated with previous therapies with linezolid.

Objectives

Here we characterize the first methicillin-linezolid-resistant (LinR) *S.epidermidis* from a patient without previous linezolid exposure.

Methods

A hypertensive 70-year-old male attended the emergency service (October-2016; Rize/Turkey; day-1) with syncope and was hospitalized. Clinical meningitis diagnosis was established in the following days but cultural+microscopic CSF analysis was negative. Antibiotic therapy was initiated and included ceftriaxone, netilmicin and vancomycin. Patient condition deteriorated and *S.epidermidis* (VitekII identification) from a hemoculture was recovered (day-14). Patient died (day-16) with multiple organ failure. Linezolid was never given to the patient and it has been scarcely used in the hospital. No other patients presented LinR-*S.epidermidis* infections. Susceptibility to linezolid and vancomycin was evaluated by microdilution, to daptomycin by Etest and to other 12 antibiotics by disk diffusion (EUCAST). The search of *cfr*/*cfr(B)*/*optrA*/*mecA*/*mecC* genes and mutations in the 23S-rRNA-V-domain were done by PCR/sequencing and clonality by MLST.

Conclusions

S.epidermidis-ST22 was resistant to linezolid (MIC≥256mg/L), cefoxitin (*mecA* gene), gentamicin, tobramycin, ciprofloxacin, chloramphenicol, erythromycin, clindamycin, tetracycline, cotrimoxazol and fusidic acid but not to quinuspristin-dalfopritin, rifampicin, tigecycline, vancomycin (MIC=4mg/L) and daptomycin (MIC=0,38mg/L). The previous identified T2530A, C2560T and the new T2834C mutations in 23S-rDNA-V-domain were detected (GenBankreference:CP000029), but not the often described G2576U or *cfr*/*optrA* genes.This is the first description of a linezolid-resistant *S.epidermidis* without previous linezolid treatment. Understanding the driving factors behind this phenomenon is critical for antimicrobial stewardship.

FEMS7-2908
Pathogens / Pathogenicity - Part II

FREQUENCY AND PROFILE OF SENSITIVITY OF MICROORGANISMS ISOLATED FROM PATIENTS CARRIED OUT AT A PRIVATE HEALTH INSTITUTION OF SAO LUIS- MA

T. Novais[1], D. Sousa[2], G. Cabral[2], S. Maia[2], N. Camelo[3], F. Moraes[3], E. Fonseca[2], A. Carvalho[1]
[1]Universidade Federal do Maranhão, Hospital Universitário, São luis, Brazil
[2]Faculdade Estacio São Luis, Curso Biomedicina, São Luís, Brazil
[3]Faculdade Estacio São Luis, Curso Enfermagem, São Luís, Brazil

Backgrounds

Hospital infections have been drawing attention in recent decades, as they represent a serious public health problem. Antibiotics are used for the treatment of these infections, but the abusive use of these drugs contributes to increase the selective pressure of these drugs, creating a favorable environment for the bacteria, facilitating their proliferation and developing bacterial resistance, even though in the presence of these drugs.

Objectives

To evaluate the frequency and sensitivity profile of microorganisms isolated from patients attended at a private health institution in São Luís - Maranhão.

Methods

It was analyzed the results of the main microorganisms isolated from clinical samples and the sensitivity profile of these pathogens from the institution's clinical laboratory database from July 2013 to 2016. For the identification of the sensitivity profile, the test was performed using the disk diffusion method according to CLSI, 2015 (Institute of Clinical and Laboratory Standards).

Conclusions

It was verified that the sector with the highest frequency of isolation of microorganisms in clinical samples was the Intensive care unit (ICU). The most frequent microorganism was Escherichia coli; The uroculture presented a higher prevalence of the clinical samples. The group of beta-lactams presented greater microbial sensitivity. However, the highest percentage of the drug isolated as sensitivity was polymyxin B. Regarding microbial resistance, a higher percentage of antibiotics in the beta - lactam group was also observed. In isolation, ampicillin was the drug that showed less efficacy against the five most frequent microorganisms.

FEMS7-2366
Pathogens / Pathogenicity - Part II

STUDY OF RESISTENCE PHENOTYPES AND GENOTYPES IN STREPTOCOCCUS AGALACTIAE ISOLATED FROM NEONATES AND ADULTS WITH INVASIVE INFECTIONS IN ARGENTINA

M.G. Novosak[1], F.J. Bobadilla[1], R.F. Derna[2], I.J. Cortese[1], M.E. Laczeski[1]
[1]Instituto de Biotecnología de la Provincia de Misiones Dra. María Ebe Recca, Microbiología, Posadas, Argentina
[2]Laboraorio Müller, Bacteriología, Encarnación, Paraguay

Backgrounds

Streptococcus agalactiae (GBS) is responsible for severe invasive infections in neonates and adults with or without underlying chronic diseases. Penicillin is the drug of election for treatment, however, in allergic to β-lactams patients, erythromycin (ERI) and clindamycin (CLI) are the recommended drugs. In the last years, increased macrolide resistance has been reported. Mechanisms include changes in target site mediated by *erm* genes leading to resistance to macrolide-lincosamides-streptogramin B (MLS_B - phenotype expressed in inducible or constitutive form) or mechanisms by efflux encoded by *mef* genes leading to macrolide resistance of 14-15 members (M phenotype). The knowledge of these phenotypes allows to adapt the treatment.

Objectives

The objective was to study the mechanisms of resistance to ERI and CLI in GBS recovered from patients with invasive diseases.

Methods

Twenty six strains isolated from blood cultures and cerebrospinal liquid from neonates and adults were investigated. MLS_B resistance phenotypes was determined by the double-disc test (D-Test) and *ermB* and *mefA* genes by PCR technique.Two GBS strains (7.69%) presented resistance phenotypes, one constitutive MLS_B phenotype and the other M phenotype. The presence of *ermB* and *mefA* genes was demostrated by PCR. This showed a correlation between the phenotype and genotype detected.

Conclusions

The results indicate the importance of D-Test technique for monitoring of the resistance to these antibiotics and to implement treatment strategies. In addition, these results confirm the presence of associated genes that can be transmitted between bacteria and disseminate the mechanisms of resistance involved.

FEMS7-1830
Pathogens / Pathogenicity - Part II

CLINICAL FORMS OF CYTOMEGALOVIRUS IN CHILDREN

A. Kushugulova[1], A. Altynbekova[2], D. Bayesheva[2], V. Chudinovskikh[2], S. Zhamukova[3], Z. Abdikadyr[2], G. Tulegenova[2]
[1]Kazakhstan Association of Human Microbiome researchers, head, Astana, Kazakhstan
[2]JSC Medical University Astana, Department of Pediatric Infectious Diseases, Astana, Kazakhstan
[3]City Children's Hospital №1, City Children's Hospital, Astana, Kazakhstan

Backgrounds

CMV infection is widespread and is characterized by diverse manifestations from asymptomatic to severe with damage to internal organs and the central nervous system

Objectives

To investigate clinical forms of CMV infections in children who had cured in City Children's Hospital №1 during the time frame 2015-1016 in Astana

Methods

It were analyzed 38 clinical cases with the diagnosis "cytomegalovirus" in children, who had the treatment during 2015-1016 in City Children's Hospital №1 in Astana. Design of research is retrospective. SPSS Statistics 20 was used for statistical analysis.

Conclusions

During examination of 38 cases, where the 38 children had treatment of CMV, the most frequent cases were in the age range 0-3 months children and represent 55,26%(n=21); at the age of 4-6 months and 7-9 month the morbidity represents the equal numbers – 21.08%(n=8); from 10 till 12 months the percentage was – 2.67%(n=1). The male represented by 52,63%(n=20) and the female by 47,37%(n=18). During examination the history cases 55,26%(n=21) had congenital generalized form of CMV; 21,05% had recurrence and 23.68%(n=9) had latent form of CMV. The presence of CMV in the body was revealed by ELISA test in 60,5 %; in 7,89% by PCR; complex ELISA+PCR represented in 7,8% and in 23,68% the presence of CMV was revealed postmortem. It should be noted that 76,32 % had congenital malformations and 55,26 % of mothers had problematical obstetric history. The minimal hospital stay represented from 1 to 7 days in 34.21% and maximal hospital stay 61-86 days in 5,26% .

Conclusion: The study found that the maximal morbidity of CMV was indicated in children from 0-3 months in 55,26%, among the clinical forms congenital generalized form is revealing more frequent and represent 55.26 %.

FEMS7-0410
Pathogens / Pathogenicity - Part II

DEVELOPMENT AND APPLICATION OF A THREE-DIMENSIONALLY DIFFERENTIATED AIRWAY EPITHELIAL CELL MODEL OF THE OVINE RESPIRATORY TRACT TO STUDY HOST-PATHOGEN INTERACTIONS

N. O'Boyle[1], *E. Sutherland*[1], *C. Berry*[2], *R. Davies*[1]
[1]*Infection- Immunity and Inflammation, Bacteriology, Glasgow, United Kingdom*
[2]*Institute of Molecular Cell and Systems Biology, Cell Engineering, Glasgow, United Kingdom*

Backgrounds

Respiratory tract infections are one of the principle causes of mortality and economic loss in the livestock industry. *Mannheimia haemolytica* is the most common bacterial pathogen associated with respiratory disease in cattle and sheep. While some information regarding the virulence factors of this organism has been obtained from large animal infections, such experiments are costly and ethically questionable. The process of airway tissue colonisation also remains poorly understood.

Objectives

Through extensive characterisation and optimisation, we aimed to develop a well-differentiated *in vitro* model of the sheep airway epithelium. This model was subsequently employed to investigate epithelial colonisation and host-specificity of a panel of *M. haemolytica* isolates.

Methods

Following growth at an air-liquid interface, optimal conditions for differentiation of airway epithelial cell (AEC) cultures were determined by carrying out extensive time-course studies and comparing a wide variety of growth parameters. Histological analysis, immunofluorescence microscopy and scanning electron microscopy confirmed that the AEC cultures contained the full repertoire of relevant cell types present in the *in vivo* airway epithelium and therefore represent a highly suitable model for infection studies. Pathogenic strains of *M. haemolytica* were capable of colonising ovine AEC cultures *in vitro* and interesting insights into temporal aspects of this process were revealed. A panel of virulent and avirulent isolates from cattle and sheep were used to demonstrate patterns of colonisation and tissue invasion that were specific to the pathogenic isolates.

Conclusions

These findings reveal previously unseen pathogenic mechanisms of this important respiratory tract pathogen.

FEMS7-3099
Pathogens / Pathogenicity - Part II

UNDERSTANDING THE MECHANISM OF ACTION OF THE PRODRUG TP053

B.S. Orena[1], L.R. Chiarelli[1], G. Mori[1], V. Makarov[2], F. Gosetti[3], M. Manfredi[3], J. Madacki[4], J. Kordulakova[4], M. Guerin[5], K. Mikušová[4], E. Marengo[3], G. Riccardi[1], M.R. Pasca[1]
[1]University of Pavia, Biology and Biotechnology "Lazzaro Spallanzani", Pavia, Italy
[2]Russian Academy of Science, 2A. N. Bakh Institute of Biochemistry-, Moscow, Russia
[3]University of Piemonte Orientale, Department of Science and Technological Innovation, Alessandria, Italy
[4]Comenius University Bratislava, Department of Biochemistry-, Bratislava, Slovak Republic
[5]CIC bioGUNE- Bizkaia Technology Park, Structural Biology Unit, Derio, Spain

Backgrounds

Tuberculosis, caused by *Mycobacterium tuberculosis*, remains one of the leading public health problems worldwide. Considering the worrying spread of *M. tuberculosis* drug-resistant strains, it is urgently needed the development of antitubercular compounds with novel mechanisms of action. The thienopirimidine (TP) derivative TP053 is a new compound active against both replicating and non-replicating *M. tuberculosis* bacilli, with an MIC *in vitro* of 0.125 µg/ml. TP053 has been characterized as a prodrug activated by the reduced form of the thioredoxin-like enzyme Rv2466c.

Objectives

The study aims to characterize the mechanism of action of the prodrug TP053.

Methods

To identify the TP053 target, several *M. tuberculosis* TP053 resistant mutants were isolated harboring the mutation L240V in Rv0579, protein with unknown function. By recombineering method, it was demonstrated that Rv0579 has a role in TP053 resistance. To verify if Rv0579 is the TP053 target, the protein was expressed and purified in *Escherichia coli*, but additional improvements are needed. To clarify the mechanism of action of TP053 and to identify its active metabolite(s), *M. tuberculosis* cellular extracts treated with TP053 were analysed by proteomic and metabolomic approaches. Moreover, to demonstrate if there is a direct interaction between the TP053 active metabolite and Rv0579, innovative click chemistry experiments are *in progress*.

Conclusions

Preliminary results obtained by proteomic and metabolomic approaches suggest that TP053 could interfere with the protein synthesis pathway. These experiments will be repeated in order to confirm the results that will be validated by genetic, microbiological and biochemical approaches.

FEMS7-0131
Pathogens / Pathogenicity - Part II

PROPIONIC ACID INDUCES RAPID EVOLUTION OF CROHN'S DISEASE ASSOCIATED AIEC

M. Ormsby[1], L. Meikle[1], A. Mcintosh[1], S. Johnson[1], R. Goldstone[2], C. Edwards[3], D. Smith[2], D. Wall[1]
[1]Infection- Immunity and Inflammation, Bacteriology, Glasgow, United Kingdom
[2]Biological Chemistry- Biophysics and Bioengineering, Heriot-Watt University, Edinburgh, United Kingdom
[3]School of Medicine, Human Nutrition, Glasgow, United Kingdom

Backgrounds

Crohn's Disease (CD) is a multi-factorial disease that occurs in genetically susceptible individuals, resulting in inflammation of the digestive tract. Concurrently, the use of antibacterial compounds, particularly short chain fatty acids (SCFAs), has increased dramatically in recent years in both agriculture and human food. One such SCFA, propionic acid (PA), inhibits *Salmonella* and *Campylobacter* colonisation of poultry. Here we show that the bacterial pathotype adherent and invasive *Escherichia coli* (AIEC), that has been isolated in increased numbers from CD patients, can use PA as a carbon source for growth.

Objectives

Our work aims to elucidate the adaptation that pre-exposure to PA may have on the ability of AIEC to colonise the human intestinal tract, outcompeting the native microflora.

Methods

Continued growth of the AIEC type strain LF82, in PA, resulted in; an increased rate of growth; greater adherence to Caco-2 intestinal epithelial cells; and a greater propensity to form biofilms under anaerobic conditions. PA use was also seen to induce shedding of inflammatory bacterial micro-compartments, with upregulation of both the *pdu* and *eut* operons, for 1,2-propanediol and ethanolamine use, respectively. Both metabolites are known to allow pathogens such as *Salmonella* Typhimurium to out-compete the native microflora during intestinal inflammation.

Conclusions

As antibiotic use has previously been implicated in the horizontal transmission of *E. coli* between poultry and humans, this work highlights the risk associated with using alternative antimicrobials such as PA in both agriculture and human food potentially evolving *E. coli* strains that are specifically adapted to life in the inflamed CD gut.

FEMS7-1565
Pathogens / Pathogenicity - Part II

AN INNOVATIVE NEXT GENERATION SEQUENCING-BASED APPRAISAL OF THE EUKARYOTIC MICROBIOME IN AUSTRALIAN TICKS

A. Paparini[1], A. Codello[1], C. Oskam[1], U. Ryan[1], P. Irwin[1]
[1]Murdoch University, Vector- and Water-Borne Pathogen Research Group School of Veterinary and Life Sciences, Murdoch WA, Australia

Backgrounds

After mosquitoes, ticks are the second most important vector of pathogens to humans, and the chief cause of vector-borne diseases in domestic animals and wildlife. Tick-borne pathogens include viruses, bacteria and protozoa of medical significance and can be successfully audited by high-throughput next generation sequencing (NGS). Tick internal tissues and organs, however, are also colonized by highly abundant groups of other symbionts. Their high dominance can hamper the detection of rare pathogens of interest, especially when universal PCR primers are used for NGS.

Objectives

We present an innovative approach to analyse the tick microbiome by selectively retrieving DNA from protozoan parasites, in the presence of highly abundant genomic DNA, from the tick and the vertebrate host. The objective was to use this approach, to audit the eukaryotic microbiome in Australian ticks, with a focus on potential pathogens.

Methods

DNA from Australian ticks of various species and genera, sex, instar, location and feeding status was processed by NGS (Miseq, Illumina). Amplicons were generated using universal PCR primers, used in conjunction with novel tick- and mammalian-blocking primers.

Conclusions

A novel approach was developed to prevent the undesired amplification of eukaryotic sequences from the tick and mammalian host. This allowed the detection, identification and quantification of various blood-borne parasites, including *Babesia*, *Theileria* and *Trypanosoma*. A number of Bacterial taxa were also identified, including two potentially new species of *Francisella* sp. Statistical analyses revealed novel insights onto the biogeography of Australian ticks and their associations with potential pathogens.

FEMS7-2023
Pathogens / Pathogenicity - Part II

EXCISION OF SALMONELLA ENTERICA SEROVAR ENTERIDIS PATHOGENICITY ISLAND ROD21 PLAYS A KEY ROLE DURING SYSTEMIC INFECTION IN MICE

C. Pardo-Roa[1,2], G.A. Salazar[1,2], L. Noguera[1,2], F.J. Salazar-Echegarai[1,2], C.A. Riedel[1,3,4], A.M. Kalergis[1,2,5,6], M. Alvarez-Lobos[1,7], S.M. Bueno[1,2,6]
[1]Milleniumm Institute on Immunology and Immunotherapy, IMII, Santiago, Chile
[2]Pontificia Universidad Católica de Chile,
Facultad de Ciencias Biológicas- Departamento de Genética Molecular y Microbiología, Santiago, Chile
[3]Universidad Andrés Bello, Facultad de Medicina, Santiago, Chile
[4]Universidad Andrés Bello, Facultad de Ciencias Biológicas-Departamento de Ciencias Biológicas, Santiago, Chile
[5]Pontificia Universidad Católica de Chile, Facultad de Medicina- Departamento de Endocrinología, Santiago, Chile
[6]INSERM, U1064, Nantes, France
[7]Pontificia Universidad Católica de Chile, Facultad de Medicina- Departamento de Gastroenterología, Santiago, Chile

Backgrounds

ROD21 is a Pathogenicity Island (PAI) present in the chromosome of *S.* Enteriditis (SEn). ROD21 harbors genes encoding virulence associated proteins and is an excisable PAI. The excision of ROD21 is a dynamic process related to environmental conditions. In mice, strains unable to excise ROD21 show reduced ability to colonize the liver and spleen. These results suggest that the excision may regulate virulence mechanisms during infection.

Objectives

To determine the effect of ROD21 excision during SEn infection *in vivo*.

Methods

C57BL/6 mice were intragastrically infected with SEn PT4 (SEn WT) or SEn strain unable to excise ROD21 (SEnDintDxis). Stomach, ileum, ceaecum, colon, mLN, blood, spleen, liver, gallbladder and feces were recovered at different times after infection **(**between 1 h and 10 days) and CFU counting and gDNA extraction were performed to evaluate bacterial load and ROD21 excision frequency, respectively. **Results:** SEn WT and SEnDintDxis were detected during the 10 days of infection in the gut. The transit of *S.* Enteritidis across the small intestine occurs rapidly for both strains, although the mutant strain was not detected in colon after 24 h of infection. SEn WT was able to colonize internal organs such as spleen, liver and gallbladder faster and more efficiently as compared to SEnDintDxis. Finally, the frequency of ROD21 excision showed differences in organs during the different phases of the infective cycle

Conclusions

Our data show that ROD21 excision is an important process to regulate SEn mechanisms involved in virulence during the systemic stages of infection.

FEMS7-0163
Pathogens / Pathogenicity - Part II

TYPE III SECRETION SYSTEM OF PSEUDOMONAS AERUGINOSA AFFECTS MUCIN GENE EXPRESSION VIA NF-KB SIGNALING IN HUMAN CARCINOMA EPITHELIAL CELLS AND A PNEUMONIA MOUSE MODEL

J.W. PARK[1], J.H. Lee[2], S.R. Oh[1], U.H. Ha[2], K.S. Ahn[1]
[1]Korea Research Institute of Bioscience & Biotechnology, Natural Medicine Research Center, Cheongju-si, Republic of Korea
[2]Korea University, Department of Biotechnology and Bioinformatics, Sejong, Republic of Korea

Backgrounds

Pseudomonas aeruginosa is a ubiquitous soil bacterium and versatile opportunistic pathogen, capable of causing life-threatening acute and chronic infections in a variety of patient populations. Many clinical isolates of P. aeruginosa have a specialized apparatus for injecting toxins into eukaryotic cells, namely, the type III secretion system (T3SS). The P. aeruginosa T3SS is a syringe-like apparatus on the bacterial surface, with 4 effector toxins: ExoS, ExoT, ExoY, and ExoU.

Objectives

Here, we investigated the effect of ExoS and ExoT of the T3SS of P. aeruginosa K strain (PAK). We aimed to investigate whether the ExoS and ExoT effector proteins of P. aeruginosa affect the expression of mucin gene via NF-κB signaling pathways.

Methods

To understand the T3SS, we used ΔExoS, ΔExoT, and ExsA::Ω mutants, as well as PAK-stimulated A549 cells. We investigated the effects of ΔExoS, ΔExoT, and ExsA::Ω on the development of pneumonia in PAK-infected mouse models. We also examined the effects of ΔExoS, ΔExoT, and ExsA::Ω on mucin gene production in A549 cells. ΔExoS and ΔExoT reduced NF-κB phosphorylation, together with mucin gene expression in PAK-infected mouse models and A549 cells.

Conclusions

To conclude, P. aeruginosa infection induced the expression of mucus gene, and P. aeruginosa T3SS appeared to be a key player in mucin gene expression, which is further controlled by NF-κB signaling. These findings might be useful in devising a novel therapeutic approach for the treatment of chronic and acute pulmonary infections.

FEMS7-0778
Pathogens / Pathogenicity - Part II

TRICHOMONAS VAGINALIS A-ACTININ 2, AN ADHESIN FOR HOST CELLS, MODULATES HOST IMMUNE RESPONSES BY INDUCING TOLEROGENIC DENDRITIC CELLS VIA IL-10 PRODUCTION FROM REGULATORY T CELLS

S.J. Park[1], H.Y. Lee[1], Z. Kim[1]
[1]Yonsei University College of Medicine, Environmental Biology, Seoul, Republic of Korea

Backgrounds

Trichomonas vaginalis is a protozoan pathogen that induces severe immune responses in hosts. *T. vaginalis* α-actinin 2, Tvα-actinin 2, has been used for diagnosis of trichomoniasis both in men and women.

Objectives

This study was undertaken to examine a role of Tvα-actinin 2 as an antigenic molecule to induce immune response from humans.

Methods

An immunofluorescence microscopy was used to observe localization of Tvα-actinin 2 upon contacts of *T. vaginalis* with vaginal epithelial cells (VEC) and prostate cell lines (RWPE-1). Antibody blocking experiments was performed to examine the role of Tvα-actinin 2 in *T. vaginalis*-host cell interaction. Flow cytometry, ligand-binding immunoblotting assay, and observation by fluorescence microscopy were used to detect the binding of Tvα-actinin 2 to these cell-lines. ELISA was employed to measure cytokine production by VEC, RWPE-1, mouse dendritic cells (DCs), or T-cells stimulated with *T. vaginalis* or Tvα-actinin 2 protein.

Conclusions

Surface localization of Tvα-actinin 2 was observed in *T. vaginalis* incubated with human epithelial cell-lines. Pretreatment of *T. vaginalis* with anti-rTvα-actinin 2 Abs resulted in reduction in cytoadherence and cytotoxicity. Direct binding of Tvα-actinin 2, especially the truncated N-terminal portion of Tvα-actinin 2 to VECs was demonstrated. Both *T. vaginalis* and rTvα-actinin 2 induced cytokine production from epithelial cell lines which includes IL-10. Moreover, CD4+CD25- regulatory T-cells (Treg cells) incubated with rTvα-actinin 2-treated DCs produced high levels of IL-10. These data suggested that α-actinin 2 plays a role in the pathogenesis of *T. vaginalis* by serving as one of the adhesins to the host cells. In addition, this surface molecule modulates immune responses via the IL-10 production by Treg cell. Therefore, *T. vaginalis* may use its surface protein, α-actinin 2 to evade excess immune responses which occurred frequently in trichomoniasis.

FEMS7-1270
Pathogens / Pathogenicity - Part II

EXPRESSION PROFILE OF EMRKY EFFLUX PUMP DURING SHIGELLA'S INTRACELLULAR LIFE

M. Pasqua[1], M. Grossi[1], L. Aussel[2], F. Barras[2], M.L. Di Martino[3], G. Prosseda[1], B. Colonna[1]
[1]Sapienza - Università di Roma, Department of Biology and Biotechnologies "C. Darwin", Roma, Italy
[2]Aix-Marseille Université - CNRS- Institut de Microbiologie de la Méditerranée, Laboratoire de Chimie Bactérienne, Marseille, France
[3]Biomedical Center- Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden

Backgrounds

In several bacterial pathogens, efflux pumps, besides exporting antimicrobial agents, play a role in bacterial pathogenicity.
Shigella, the causative agent of bacillary dysentery, has conserved 14 out of 20 operons encoding efflux pumps systems present in the genome of its commensal ancestor *Escherichia coli*.

Objectives

The aim of this work was (i) to analyze the regulation of *Shigella's* efflux pumps during the infection of macrophages and epithelial cells and (ii) to identify and characterize those that are required for bacterial pathogenesis.

Methods

The expression of efflux pumps was monitored by qRT-PCR during infection of macrophages and epithelial cells. Strains defective for efflux pump encoding genes were used in infection assays to investigate the existence of interplays between them. Competition assays were conducted to compare the importance of these genes in *Shigella*'s fitness. To better understand the regulative network, transcriptional fusions were monitored in the presence of different stimuli which reflect the intracellular environment.

Conclusions

Our results allowed us to identify efflux pump encoding genes up- or downregulated in macrophages and epithelial cells. Among them, *emrK*, encoding the membrane subunit of the EmrKY multidrug efflux pump, was specifically up-regulated within macrophages. Moreover, an *emrK* mutation was shown to affect *Shigella*'s fitness.
Our observations also reveal that its role, still unknown, and its modulation during infection are dependent upon pH and ion concentrations.

FEMS7-2610
Pathogens / Pathogenicity - Part II

NASAL SELF-ADJUVANTED VACCINE AGAINST SHIGELLA FLEXNERI

Y. Pastor[1], A.I. Camacho[1], A.G. Gil[2], R. Ramos[3], A. López de Ceráin[2], I. Peñuelas[3], J.M. Irache[4], C. Gamazo[1]
[1]Universidad de Navarra, Microbiology, Pamplona, Spain
[2]Universidad de Navarra, Toxicology, Pamplona, Spain
[3]Clínica Universidad de Navarra, Radiopharmacy, Pamplona, Spain
[4]Universidad de Navarra, Pharmacy and Pharmaceutical technology, Pamplona, Spain

Backgrounds

Shigellosis is one of the leading causes of diarrhea in developing countries. *Shigella flexneri* is estimated to cause more than 80 million dysenterial episodes each year but no vaccine is available yet. Since non-living vaccines seem to be the safest option, our group has focused on liposome-like outer membrane vesicles.

Objectives

The aim was to improve the yield and safety in the isolation process of the outer membrane vesicles, and evaluate toxicity, biodistribution and protection after nasal administration in mice.

Methods

Outer membrane vesicles were obtained by two different methods: either from the supernatant of growing bacteria (OMV), or from bacteria treated at 100 ºC, 15 min (HT). Cytotoxicity and immunostimulating properties were studied in Raw 264.7 macrophage cell line *in vitro.* Acute and chronic toxicity studies and biodistribution studies were performed in rat and mice, respectively. Finally, protection against an infection with *S. flexneri* was evaluated in mice.

Conclusions

Heat treatment simultaneously achieves the inactivation of bacteria, leading a safe working process, as well as an increase in the yield the vaccine product (HT) in four times with respect OMV. In addition, HT appeared to be non-cytotoxic, induced expression of main differentiation markers of antigen presenting cells and did not elicit adverse effects *in vivo*. Finally, the HT vaccine product was as effective as OMV in protection against an experimental infection with *S. flexneri* in mice. These findings support the use of the new HT product as a new vaccinal candidate to face shigellosis.

FEMS7-1505
Pathogens / Pathogenicity - Part II

TLRS DIFFERENTIALLY INDUCE IG SYNTHESIS FOLLOWING PAMPS STIMULATION IN THE CARP CATLA CATLA BY ACTIVATING MAPK AND NF-KB SIGNALING CASCADE

B. Patel[1], M. Samanta[2], S. Das[3]
[1]National Institute of Technology, Life Science, Rourkela, India
[2]ICAR-Central Institute of Freshwater Aquaculture, Fish Health Management Division, Bhubaneswar, India
[3]National Institute of Technology- Rourkela, Life Science, Rourkela, India

Backgrounds

The extracellular-signal regulated kinases have emerged as highly conserved signal transducers of mitogen activated protein kinase family across vertebrates. These serine/threonine kinases participate in propagating intracellular signals initiated by ligand stimulated cellular receptors to transcription factors eliciting cytokine release. Although ERK signaling has been extensively studied in mammalian counterparts, very little is known about its existence in carps and its role in augmentation of Ig synthesis.

Objectives

To gain insights into the efficacy of MAPkinase cascade in orchestrating fish antigen receptor generation, *Catla catla* fingerlings were induced with various TLR agonists or pathogen associated molecular patterns (PAMPs).

Methods

Analysis of upstream signaling events revealed that PAMPS stimulated tissues led to a significant upregulation ($P<0.001$, Two-way ANOVA) of different TLRs (TLR2, TLR3, TLR4 and TLR5) followed by activation of MyD88 dependent and independent pathway. Consequent activation of ERK, triggered NF-kB mediated cytokine production and enhanced expression of circulating IgZ and IgM in a time dependent manner as was evident by qRTPCR analysis, flow cytometry and ELISA. Pretreatment with ERK inhibitor (UO126) antagonized PAMPs mediated TLR stimulation leading to sequential downregulation of MyD88/NF-kB/cytokines via interrupting ERK/NF-kB signaling axis.

Conclusions

Together these results demonstrate that TLR triggering stimulates IgZ and IgM production via activation of ERK and NF-kB in *C. catla* indicating that NF-kB mediated cytokine production and ERK1/2 signaling is not only functional in fish, but may be crucial for generation of Ig repertoire in lower vertebrates.

FEMS7-1064
Pathogens / Pathogenicity - Part II

CHARACTERIZATION OF THE LUNG MICROBIOME IN LEGIONELLA-ASSOCIATED PNEUMONIA AND ITS EVOLUTION DURING ANTIBIOTIC TREATMENT

A.E. Pérez Cobas[1,2], C. Ginevra[3,4], C. Rusniok[1,2], J. Sophie[3,4], C. Buchrieser[1,2]
[1]Institute Pasteur, Biology of Intracellular Bacteria, Paris, France
[2]CNRS UMR 3525, 75724, Paris, France
[3]Centre National de Référence des Légionelles, Hospices Civils de Lyon, Lyon, France
[4]Centre International de Recherche en Infectiologie- Université Lyon 1- UMR CNRS 5308- U1111 Ins erm, Ecole Normale Supérieure de Lyon, Lyon, France

Backgrounds

Pneumonia represents a major public health problem with a high rate of morbidity and mortality worldwide. Despite the explosion of microbiome (MC) analyses, very little is known about the lung MC, especially with respect to bacterial pneumonia. The lung MC seems to contribute to the stimulation of the immune system and to inflammation. It thus plays possibly a role in the response to lung infections.

Objectives

Here we characterized the dynamics of the lung MC during bacterial infection and antibiotic therapy.

Methods

We collected broncheoalveolar samples from patients with pneumonia caused by *Legionella pneumophila* during around two months of hospitalisation and performed a longitudinal analysis of their lung MC. We characterized the bacterial and fungal diversity of these samples by deep sequencing (Illumina) of the 16S rRNA gene (bacteria) and the ITS region (fungi).

Conclusions

During infection the bacterial fraction of the lung MC was characterized by a low diversity and a dominance of the pathogen, as *L. pneumophila* represented more than 50% of the identified bacteria. Antibiotic treatment led to a strong change in the MC as we observed a marked decrease in the abundance of *L. pneumophila* and an increase in the bacterial diversity. The commonly identified bacteria of the lungs such as *Prevotella*, *Fusobacterium* or *Staphylococcus* were recovered. In contrast, the composition and diversity of the fungal fraction was more homogeneous. However, the fungal diversity was higher during infection probably because of a colonization advantage of fungi due to the loss of the bacterial MC during antibiotics treatment.

FEMS7-3014
Pathogens / Pathogenicity - Part II

PSEUDOMONAS PROTEGENS AND PSEUDOMONAS CHLORORAPHIS: SWITCH BETWEEN ROOT- AND INSECT-ASSOCIATED LIFESTYLES

P. Vesga[1], P. Flury[1], M. Péchy-Tarr[2], C. Keel[2], M. Maurhofer[1]
[1]Institute of Integrative Biology- Federal Institute of Technology Zurich ETHZ, Plant Pathology, Zurich, Switzerland
[2]University of Lausanne, Fundamental Microbiology, Lausanne, Switzerland

Backgrounds

Insect pests are a major agricultural problem and very difficult to control. Root-colonizing fluorescent pseudomonads are known for their ability to promote plant growth and to protect plants against soilborne fungal pathogens, but some of them belonging to the species *Pseudomonas protegens* and *P. chlororaphis* additionally have insecticidal activity. They can colonize the gut of insect larvae and invade the hemocoel causing systemic infection and death. These bacteria produce an insect toxin termed Fit, which contributes to insecticidal activity.

Objectives

To answer the question whether *P. protegens* and *P. chlororaphis*, which were so far thought to be mostly associated with plants and soil, are naturally associated with insects.

Methods

In order to answer this question, bacteria were isolated from insects collected from the field. Isolates were assessed for presence of the Fit insect toxin gene and insecticidal activity and were phylogenetically characterized. So far, we found that Fit-harbouring pseudomonads are common in different insect species, which indicates that insects are an additional ecological niche for *P. protegens* and *P. chlororaphis*. Currently, we are performing a RNA-sequencing analysis with *P. protegens* CHA0 to determine the genes it needs to switch between a root- and an insect-associated lifestyle. In addition, we have demonstrated, in insect backgrounds, the expression of known insecticidal factors as well as factors involved in suppression of plant pathogens and competition.

Conclusions

Altogether, this knowledge will contribute to a better understanding of *Pseudomonas*-insect interactions and to develop future biocontrol strategies for insects on top of diseases.

FEMS7-3265
Pathogens / Pathogenicity - Part III

HOST-MICROBIOTA INTERACTIONS IN INFLAMMATORY ARTHRITIS

L. Lam[1], D. Regan-Komito[1], J. Faith[2], T. Griseri[1], F. Powrie[1]
[1]University of Oxford, Kennedy Institute of Rheumatology, Oxford, United Kingdom
[2]Mount Sinai School of Medicine, Genetics and Genomic Sciences, New York, USA

Backgrounds

The gut is the primary site of host-commensal symbiosis, but disequilibrium can drive inflammatory disease and damaging systemic immune responses. Spondyloarthropathies are a form of inflammatory arthritis, the most common of which is ankylosing spondylitis (AS), characterized by joint deformation and fusion throughout the body. Intriguingly, over 70% of AS patients have subclinical intestinal inflammation and 15-25% develop inflammatory bowel disease. Additionally, arthritis is the most common extraintestinal manifestation of IBD. Together, these findings suggest a gut-joint connection in AS pathogenesis.

Objectives

While microbial dysbiosis is strongly associated with inflammatory arthritis, it is unclear how altered intestinal community composition modulates systemic immune responses. IgA secreted at mucosal surfaces coats different species of intestinal bacteria and is a critical mediator of homeostasis. While commensal microbiota may be coated with IgA, pathogens elicit high-affinity responses, making IgA coating a powerful metric for identifying specific bacteria that trigger inflammation.

Methods

The proposed research will use differential immunoglobulin A coating of fecal bacteria coupled with 16S rRNA sequencing (IgA-SEQ) to identify immunopathogenic members of the microbiota. IgA+ and IgA- bacteria from will be collected by fluorescence-activated cell sorting in both ankylosing spondylitis (AS) patients and healthy controls.

Conclusions

In parallel with IgA-seq, patient libraries of bacterial isolates will be generated using high-throughput cultivation platforms. To identify immunomodulatory microbes, IgA+ and IgA- bacterial consortia will be screened in gnotobiotic mouse models of AS. This work has the potential to identify host-microbiota interactions that drive local intestinal inflammation and systemic joint disease.

FEMS7-0006
Pathogens / Pathogenicity - Part III

CHEMICAL COMPOSITION, ANTICANDIDAL AND ANTIOXIDANT ACTIVITY OF STAR ANISE ESSENTIAL OIL

S. Patil[1]
[1]*Shri Shivaji Science College- Amravati, Department of Microbiology & Biotechnology, Amravati, India*

Backgrounds

Plant based medicines are in use ever since the antiquity and still a large population of the world is relying on the herbal based products in controlling the various ailments. Star anise is the dried, star shaped fruit of *Illicium verum* and is indigenous to South Eastern China and also produced to a small extent in India. Star anise essential oil (EO) is used as a fragrance in soaps, cosmetics, perfumes, and toothpaste, and to mask undesirable odors in drug products.

Objectives

The objective of the present study was to evaluate the antimicrobial potential of star anise essential oil against *Candida* species along with chemical constituents, free radical scavenging potential and possible synergism with commonly used antifungal drugs.

Methods

Sensitivity of 50 clinical isolates of *Candida* to star anise oil was determined by agar disc diffusion method. Minimum inhibitory concentrations (MIC) were evaluated by broth microdilution method. GC-MS analysis of essential oil components was performed by using JEOL, GC-MS, Japan. The synergic efficacy of antifungal drugs with EO was assessed by using checkerboard assay.

Conclusions

Majority of *C. albicans* and *C. glabrata* isolates were sensitive to star anise EO at all the concentrations tested. The broth microdilution assay gave MIC values ranging from 1.25- >5.00 µg/ml and *C. krusei* exhibited higher sensitivity to star anise oil than other isolates with mean MIC of 1.86 µg/ml. GC-MS studies showed presence of *trans*-anethole (22.34%), limonene (14.63%), α-trans-bergamotene (14.28%), estragole (12.24%), a-cubebene (10.86%). However, a-pinene (9.57%), and 4-terpineol (8.84%) were present comparatively in lower quantities. At 500 µg/ml concentrations the scavenging activity of both star anise EO and ascorbic acid was same (8.68%). The combinational effects of EOs with antifungal drugs showed varying degrees of interactions. The results suggest that star anise oil possess active anticandidal components and synergistic interactions for the management of fungal infections.

FEMS7-0890
Pathogens / Pathogenicity - Part III

SURVIVAL OF RESISTANCE PLASMID IN A BACTERIAL POPULATION EXPOSED TO PLASMID-DEPENDENT PHAGES, PREDATORS AND/OR ANTIBIOTICS

T. Patinen[1], J. Cairns[2], R. Penttinen[1], T. Hiltunen[2], M. Jalasvuori[1]
[1]University of Jyväskylä, The Department of Biological and Environmental Science, Jyväskylä, Finland
[2]University of Helsinki,
Department of Food and Environmental Sciences/Microbiology and Biotechnology, Helsinki, Finland

Backgrounds

Resistance plasmids are common among hospital bacterial pathogens, therefore horizontal gene transfer as mediated by these mobile elements plays a critical role in the evolution of antibiotic resistance.

Objectives

In this study, we set to investigate how predation, plasmid-depended bacteriophages and antibiotics in both inhibiting and sub-inhibiting concentrations affects the persistence of plasmids in bacterial populations.

Methods

Bacteria carrying a resistance conferring conjugative plasmid were let to interact in daily refreshed cultures for 50 days in a factorial experiment setup containing (A) predator Tetrahymena thermophila, (B) plasmid-dependent phage PRD1 and (C) aminoglycosides and beta-lactams. Evolved populations were studied for their presence of plasmids as well as for various other phenotypic attributes.

Conclusions

Plasmid-dependent phages are effective in removing antibiotic resistance plasmids from bacteria. Interestingly, conjugation appears to be necessary for a plasmid to maintain itself in a bacterial population under predation. On the other hand, simultaneous exposure to phages and antibiotics cause plasmids to become conjugation defective. These results suggest that various biological interactions play a crucial role in determining whether bacteria retain their plasmids.

FEMS7-1532
Pathogens / Pathogenicity - Part III

UNIQUE STRUCTURAL FEATURES REVEAL THE BINDING MECHANISM FOR A LARGE FAMILY OF BACTERIAL HOST CELL ADHESINS

J. Paxman[1], A. Lo[2], S. Panjikar[3], M. Kuiper[4], S. Nocadello[5], D. Moriel[2], C. Gee[3], M. Schembri[2], B. Heras[1]
[1]La Trobe Institute for Molecular Science, Biochemistry and Genetics, Melbourne, Australia
[2]The University of Queensland, Australian Infectious Diseases Research Centre, Brisbane, Australia
[3]Australian Synchrotron, Macromolecular Crystallography, Melbourne, Australia
[4]The University of Melbourne, Victorian Life Sciences Computation Initiative, Melbourne, Australia
[5]Northwestern University, Department of Biochemistry and Molecular Genetics, Chicago, USA

Backgrounds

Autotransporters (type V secretion pathway) are the largest family of outer membrane and secreted proteins in Gram-negative bacteria. Despite their prevalence, there are few crystal structures of autotransporters and their definite mechanisms of action remain largely unknown. Most autotransporters are associated with colonisation for many medically important bacterial pathogens. Their β-helical structures are thought to remain associated with the bacterial cell surface where they function as adhesins.

Objectives

We have investigated the UpaB autotransporter adhesin that is critical for uropathogenic *E. coli* (UPEC) colonisation of the urinary tract[1].

Methods

Determination of the UpaB crystal structure revealed a significant deviation from previous autotransporter β-helical structures[2], to accommodate two very different host cell binding sites. We found that the UpaB β-helix forms a long deep groove to interact with epithelial glycosaminoglycans. The second UpaB binding site was found to form a completely new type of interaction with human fibronectin. This is very significant considering that most of the 100 bacterial fibronectin binding proteins follow a single and very different type of interaction with fibronectin. Following initial attachment of UPEC to the urinary tract by fimbrial adhesins, the UpaB binding activities likely work in unison to promote more intimate and higher affinity interactions at a later stage of colonisation.

Conclusions

We are the first to show how the large autotransporter family interacts with host cell surfaces to facilitate bacterial colonisation/pathogenesis. The unique UpaB structure also reveals unprecedented structural plasticity in the autotransporter β-helices, which should lead to a re-evaluation of the current autotransporter classification.

FEMS7-0614
Pathogens / Pathogenicity - Part III

DAM OVEREXPRESSION IMPACTS MOTILITY AND VIRULENCE OF THE ENTOMOPATHOGENIC BACTERIUM PHOTORHABDUS LUMINESCENS

A. Payelleville[1], A. Givaudan[1], J. Brillard[1]
[1]Université de Montpellier, Diversité Génome Interactions Microorganismes Insectes DGIMI, Montpellier cedex 5, France

Backgrounds

Photorhabdus luminescens is an entomopathogenic bacterium symbiotically associated with nematodes of the genus *Heterorhabditis*. The nemato-bacterial complex is able to kill a broad range of insect's crop pest and is therefore use in biocontrol. Dam is the most described DNA-methyltransferase and is widespread in gamma-proteobacteria. Dam DNA methylation can play a role in gene expression and in virulence of several bacterial species but has never been studied in *P. luminescens*.

Objectives

We studied the functionality of a *dam* ortholog identified in *P. luminescens* and its involvement in bacterial phenotypes and virulence.

Methods

The *dam* gene was cloned on a plasmid under the control of a strong promoter. This construction complemented DNA methylation of an *E. coli dam* mutant. It also allowed the overexpression of Dam protein in *P. luminescens* and different phenotypes were assayed.

Conclusions

Overexpression of Dam protein in *P. luminescens* did not impair growth ability nor mutation rates relative to a control harboring an empty plasmid. In contrast, it drastically reduced swimming motility. Similarly, *dam* overexpression in the closely related bacterium *Xenorhabdus nematophila* also impaired motility. In addition, the *dam*-overexpressing *P. luminescens* strain showed a delayed virulence compared to that of the control strain after injection in larvae of the lepidopteran *Spodoptera littoralis*. These results reveal that Dam plays a major role during *P. luminescens* insect infection. To go further, the analysis of the DNA methylation pattern of the whole *P. luminescens* genome will be performed by SMRT sequencing.

FEMS7-0895
Pathogens / Pathogenicity - Part III

MULTILOCUS SEQUENCE TYPES PREDICT PHYLOGENY AND GENE CONTENT BUT NOT ISOLATION SOURCE FOR BURKHOLDERIA MULTIVORANS

C. Peeters[1], V.S. Cooper[2], P.J. Hatcher[3], B. Verheyde[1], A. Carlier[1], P. Vandamme[1]
[1]Ghent University, Laboratory of Microbiology - Department of Biochemistry and Microbiology, Gent, Belgium
[2]University of Pittsburgh School of Medicine, Department of Microbiology and Molecular Genetics, Pittsburgh, USA
[3]University of New Hampshire, Department of Computer Science, Durham, USA

Backgrounds

Burkholderia multivorans is an important pathogen in people with cystic fibrosis (CF) in many countries including Belgium, and the continued emergence of unique *B. multivorans* strains (as defined by multilocus sequence typing) in CF patients suggests acquisition from nonhuman sources, such as the natural environment. Because environmental pressure can select for traits that confer virulence, the natural environment may serve as a reservoir of opportunistic pathogens.

Objectives

The aim of the present study was to examine whether the isolation source (CF or environment) or the multilocus sequence type (ST) of *B. multivorans* better predicted their genomic content and functionality.

Methods

We identified four pairs of *B. multivorans* isolates, representing distinct STs and consisting of one CF and one environmental isolate. All genomes were sequenced using the PacBio SMRT sequencing technology, which resulted in eight high-quality *B. multivorans* genome assemblies.

Conclusions

The present study demonstrated that the genomic structure of *B. multivorans* is highly conserved and that the *B. multivorans* genomic lineages are defined by their ST. Orthologous protein families were not uniformly distributed among chromosomes, with core orthologs being enriched on the primary chromosome and ST-specific orthologs being enriched on the second and third chromosome. The ST-specific orthologs were enriched in genes involved in defense mechanisms and secondary metabolism, corroborating the strain-specificity of these virulence characteristics. Finally, the same *B. multivorans* genomic lineages were isolated from CF and environmental samples and on different continents many years apart, demonstrating the evolutionary persistence and ubiquity of these strains in different niches and on different continents.

FEMS7-2091
Pathogens / Pathogenicity - Part III

DECTIN-1 PLAYS A CRITICAL ROLE DURING THE MICROGLIAL PHAGOCYTOSIS OF THE FILAMENTOUS PATHOGENIC FUNGUS LOMENTOSPORA PROLIFICANS

A. Pellon[1], A. Ramirez-Garcia[1], I. Buldain[1], A. Antoran[1], A. Arbizu-Delgado[1], C. Matute[2], A. Rementeria[1], F.L. Hernando[1]
[1]University of the Basque Country UPV/EHU,
Fungal and Bacterial Biomics Research Group. Dept. of Immunology- Microbiology & Parasitology, Leioa, Spain
[2]University of the Basque Country UPV/EHU, Neuroscience, Leioa, Spain

Backgrounds

The filamentous ascomycete *Lomentospora* (*Scedosporium*) *prolificans* is an emerging pathogen that is able to invade the Central Nervous System (CNS). Unfortunately, little is known about its neurotropism and how microglia, the resident immune cells in the CNS, manages these infections.

Objectives

To study the interactions between *L. prolificans* and microglia, and to analyze the role of the macrophage receptors Dectin-1 (Dc-1) and Mannose Receptor (MR) in this interplay.

Methods

Conidia were co-cultivated with two microglial cellular models, the BV-2 cell line and primary cultures, or with J774A.1 monocytes. Then, we characterized these co-cultures by measuring phagocytosis and survival rates, production of reactive oxygen and nitrogen species (ROS and RNS), and pro-inflammatory cytokines. Moreover, the role of Dc-1 and MR was studied using specific inhibitors.

Conclusions

We showed that both microglial models were able to phagocytize *L. prolificans* but in very low rates, being microglial survival rates also low. Additionally, TNF-α, IL-6, and ROS production was also deficient in microglia. Finally, we observed that Dc-1 was the most important since its inhibition blocked up to 84% of the phagocytic events, while only 71% inhibition was induced when MR was blocked. In summary, this study delves into *L. prolificans* pathobiology, and shed light on its neurotropism. This process might be, at least in part, a consequence of microglial deficiencies, contributing to the fatal outcomes of these mycoses.

Funding: GIU15/36 from the Basque Government. AP (postdoctoral), IB and AA (predoctoral) are recipients of fellowships from the UPV/EHU or the Basque Government, respectively.

FEMS7-2419
Pathogens / Pathogenicity - Part III

INDUCED REARRANGEMENT OF PASA5 PLASMID IN AEROMONAS SALMONICIDA

R. Farto[1], L. Rivera[1], N. Serrano[1], T. Pérez Nieto[1]
[1]Universidad de Vigo, Biología Funcional y Ciencias de la Salud, Vigo, Spain

Backgrounds

Quorum system (QS) appears to be involved in regulating expression of Type Three Secretion System (T3SS). However, it has not been confirmed its direct association with the regulation of specific genes of this system associated with virulence such as *ascC* or *ascV*.

Objectives

The main purpose of this study was to give a broader understanding on how QS system AsaI/R, in *A. salmonicida* subspecies *salmonicida*, influences the expression of T3SS.

Methods

The effect of *asaR* and *asaI* genes on expression of T3SS genes were studied by qPCR in the strain RIM 33.1, its mutants and complemented strains for genes of QS system AsaI/R, and control strains.

Conclusions

The *asaR* gene activated the expression of *ascV*, this regulation might also occurs for *ascC.* On the other hand, *asaI*, could be also causing activation of them. However the regulatory role was not confirmed, since absence of expression was detected in complemented or control strains. Negative amplification for *ascC* and *ascV* and other genes of T3SS locus (*ati2, exsD, aopN*) was detected by PCR; by contrast it was positive for genes located upstream and downstream of T3SS locus (*traD*, *aopO*), showing the loss of T3SS locus, but not the pAsa5 plasmid.

Recent studies have shown that insertion sequences, which are abundant in *A. salmonicida* pAsa5 plasmid, are involved in rearrangement events that lead to the loss of *ascC* and *ascV* genes or other changes also in the chromosome. Since, we are introducing a new plasmid in the cell, it induced interactions with pAsa5 plasmid being affected expression of genes.

FEMS7-1190
Pathogens / Pathogenicity - Part III

DE NOVO ANALYSIS OF THE HAUSTORIAL TRANSCRIPTOME OF THE CUCURBIT POWDERY MILDEW FUNGUS PODOSPHAERA XANTHII REVEALS NEW CANDIDATE SECRETED EFFECTOR PROTEINS

A. Polonio[1], P. Seoane[2], A. de Vicente[1], A. Pérez-García[1]
[1]Universidad de Málaga, Microbiología, Málaga, Spain
[2]Universidad de Málaga, Plataforma Andaluza de Bioinformática, Málaga, Spain

Backgrounds

Cucurbit crops are affected, among other pathogens, by the obligate biotrophic fungus *Podosphaera xanthii*, the main causal agent of powdery mildew in cucurbits. This fungus develops a specialized structure of parasitism termed haustorium. Haustoria are developed into epidermal cells and are responsible for nutrients uptake and effectors delivery.

Objectives

The aim of this study was to obtain the haustorial transcriptome of *P. xanthii* to complete the panel of effector candidates of this fungal pathogen.

Methods

To obtain the haustorial transcriptome, we have developed an effective method for isolation of haustoria without contaminants by flow cytometry. The cDNA library was built using a combination of dT primers and random primers followed by a depletion of ribosomal sequences. Sequencing was carried out by Illumina NextSeq550.

Conclusions

After bioinformatic analysis, we were able to identify 25 new effector candidates secreted by the classic pathway (with signal peptide) and 269 new candidates secreted by the non-classic pathway (without signal peptide). Most proteins had no functional annotation. By protein modelling and ligand predictions, we are now being able to assign putative functions to some of these candidates to select those with potential roles in pathogenesis for subsequent functional *in vivo* analysis by HIGS (host-induced gene silencing). By these approaches, we are starting to shed some light into the molecular mechanisms of pathogenesis in this very important pathogen of cucurbits.

This work was supported by a grant from the Ministerio de Economía y Competitividad (AGL2013-41938-R), co-financed with FEDER funds (EU).

FEMS7-0855
Pathogens / Pathogenicity - Part III

CARBAPENEMASE GENES AND CLONAL RELATEDNESS OF CARBAPENEM RESISTANT KLEBSIELLA PNEUMONIAE CLINICAL ISOLATES FROM UNIVERSITY HOSPITAL IN NORTHERN THAILAND

K. Phannachet[1], *N. Khamprom*[1], *M. Deeudom*[1]
[1]*Chiang Mai University, Microbiology, Muang, Thailand*

Backgrounds

During April 2014 to December 2015, clinical isolates of *Klebsiella pneumoniae* were collected from patients in university hospital in Northern Thailand. The results of imipenem, meropenem and ertapenem disc diffusion tests and minimal inhibitory concentration of meropenem indicated that 23 isolates were carbapenem resistant *K. pneumoniae.*

Objectives

The aim of this study is to identify carbapenemase genes, determine carbapenemase expression and investigate clonality of carbapenemase gene-harboring *K. pneumoniae* clinical isolates from university hospital in Northern Thailand.

Methods

The identification of carbapenemase genes, bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{KPC} and $bla_{OXA48\text{-}like}$, in 23 isolates of *K. pneumoniae* was done by multiplex PCR. The expression of carbapenemase was determined by using modified carbaNP method. The clonal relatedness of carbapenemase gene-harboring isolates was performed by repetitive extragenic palindromic-PCR (rep-PCR) using $(GTG)_5$ primer and interpreted based on >80% similarity index as a cut off.

Conclusions

Among 23 isolates of carbapenem resistant *K. pneumoniae*, carbapenemase genes were identified in 19 isolates in which bla_{NDM} and $bla_{OXA48\text{-}like}$ were found in 6 and 9 isolates, respectively. Furthermore, 2 isolates of *K. pneumoniae* co-harboring bla_{NDM} and $bla_{OXA48\text{-}like}$ genes and 2 isolates co-harboring bla_{IMP} and $bla_{OXA48\text{-}like}$ genes identified in this study has not been reported in Thailand. Carbapenemase production determined by modified carbaNP revealed that 14 isolates expressed carbapenemase whereas 5 isolates were negative for this test. Clonality study showed that *K. pneumoniae* carried carbapenemase genes were classified in 14 distinct clusters.

FEMS7-0209
Pathogens / Pathogenicity - Part III

EVALUATION OF THE ANTIMICROBIAL POTENTIAL OF THE ETHANOLIC EXTRACT OF PIPER UMBELLATUM

V. Greatti[1], R. Sorrechia[1], R. Pietro[1]
[1]School of Pharmaceutical Sciences- São Paulo State University´UNESP, Drugs and Medicines, Araraquara, Brazil

Backgrounds

Piper umbellatum is popularly known as Pariparoba and has a secondary metabolite with expressive antimicrobial capacity, 4-Nerolidylcatechol. Among microorganisms that cause infections, there are *Candida krusei*, *Enterococcus faecalis* and *Escherichia coli* that have several virulence factors, causing difficulties of treatment. On the other hand, marine microorganisms are of great interest, since they are a source of new compounds, however, they are still unknown as to their antimicrobial activities.

Objectives

The aim of this study was to search for new potential antimicrobial agents derived from *P umbelatum* against pathogenic microorganisms and marine organisms.

Methods

Candida krusei (ATCC 6258), *Escherichia coli* (ATCC 25922), *Bacilus antrophaeus* (ATCC 9372), *Enterococcus faecalis* (ATCC 29212) and two marine fungi (F12, F22) were evaluated against the ethanolic extract 70% of the aerial parts of *P umbelatum*. Minimum inhibitory concentrations (MIC) were determined by the microdilution technique. The controls were amphotericin B and fluconazole (initial concentrations of 16 and 640 µg/mL) respectively for fungi and yeast; and ampicillin (initial concentration of 12.5 µg/mL) for bacteria. The extract was used at the initial concentration of 1250 µg/mL. The results showed that the ethanolic extract had a higher activity against *B. antrophaeus* at MIC of 156.25 µg/mL followed by *C. krusei* at 312.5 µg/mL and *E. faecalis* at 625 µg/mL while the others microorganisms presented MICs of 1250 µg/mL. The marine fungi evaluated in this study showed resistance to fluconazole.

Conclusions

The extract of *P. umbellatum* shows great potential as source of new molecules with antimicrobial activity.

FEMS7-3247
Pathogens / Pathogenicity - Part III

EVALUATION OF ALTERNATIVE IAA BIOSYNTHETIC PATHWAYS IN THE BACTERIA FROM THE PSEUDOMONAS SYRINGAE COMPLEX

A. Pintado[1], *I. Pérez-Martínez*[1], *M. Cerezo*[2], *V. Flors*[2], *C. Ramos*[1]
[1]*Universidad de Málaga, Biología Celular- Genética y Fisiología Animal, Málaga, Spain*
[2]*Universitat Jaume I, Departament de Ciències Agràries i del Medi Natural, Castelló de la Plana, Spain*

Backgrounds

Indole-3-acetic acid (IAA) is a phytohormone belonging to the auxin group which production is widely distributed among plant-associated bacteria. In phytopathogenic bacteria, several IAA biosynthetic pathways have been described. The best characterized is the indole-3-acetamide (IAM) pathway, where tryptophan is initially converted into IAM by a monooxygenase (*iaaM* gene), and later transformed to IAA in a reaction catalysed by a hydrolase (*iaaH* gene). *Pseudomonas savastanoi* pv. savastanoi NCPPB 3335 (Psv), which synthesizes IAA through IAM, encodes two paralogs of these two genes organized in two operons (*iaaMH-1* and *iaaMH-2*). Previously, we have demonstrated that a Psv mutant in the *iaaMH-1* operon produces an amount of IAA significantly lower than that synthesized by the wild type strain. This strain, shows a reduced virulence in olive plants. In contrast, a mutant in the *iaaMH-2* operon (which encodes a *iaaM-2* pseudogene), produces IAA levels similar to those of the wild type strain and is not affected in virulence. Unexpectedly, the *iaaMH-1* mutant and the double mutant *iaaMH-1/iaaMH-2* synthesize a residual amount of IAA, suggesting the existence of an alternative route for the production of this compound in Psv.

Objectives

Identification of the alternative IAA biosynthetic pathway active in the Psv mutant devoid of the IAM pathway.

Determination of the IAA biosynthetic pathway encoded by other strains of the *P. syringae* complex with different host spectrum.

Methods

Candidate genes search, directed mutagenesis and functional analysis.

Conclusions

Besides the IAM pathway, bacteria from the *P. syringae* complex encode other pathways for the biosynthesis of IAA.

FEMS7-2160
Pathogens / Pathogenicity - Part III

PROTEOMICS AND METABOLOMICS TOWARDS A BETTER UNDERSTANDING OF VIRULENCE OF STREPTOMYCES SCABIES, THE PLANT PATHOGEN

S. Planckaert[1]
[1]*Ghent University, WE-10, Gent, Belgium*

Backgrounds

Streptomyces species are known for their ability to produce a variety of medically and agriculturally important secondary metabolites. Unfortunately, several species are plant pathogens. *Streptomyces scabies* is recognized as the main causal agent of common scab. This plant disease affects root and tuber crops, especially potatoes. The symptoms are variable and can range from corky surface lesions to deep pits. Scab lesions are aesthetic unappealing, thereby reducing the marketability of both fresh-market and processed potatoes.

The actual causative of the disease is the secondary metabolite thaxtomin A, an inhibitor of plant cellulose biosynthesis. The genes responsible for the thaxtomin A biosynthesis are located on a pathogenicity island. Expression of these genes is mainly controlled by the TxtR regulator which is responsive to short cello-oligosaccharides, but recently other regulators are discovered (Bignell et al 2014). Also CebR, a cellobiose sensor, seems to be important in the pathogenicity of *Streptomyces scabies.* The details of this complex regulation are not fully understood.

Objectives

The combination of proteomics (LC-MS^E and MRM) and metabolomics should provide a solid basis for the further elucidation of the complex virulence mechanism of *Streptomyce*s scabies. The determination of proteins involved in virulence and their corresponding known or unknown metabolites could lead to a better understanding of *Streptomyce*s scabies' pathogenicity.

Methods

Label-free proteomics (LC-MS^E)
Targeted proteomics (LC-MRM)
Targeted metabolomics (LC-MRM)

Conclusions

The combination of proteomics (both discovery as targeted) and metabolomics reveals new insights in the regulation mechanism of the secondary metabolites involved in the virulence of Streptomyces scabies.

FEMS7-2719
Pathogens / Pathogenicity - Part III

INVESTIGATING FLAVESCENCE DOREE PHYTOPLASMA EMERGENCE AND THE PATHS OF SPREAD BY MLST IN CROATIA

J. Plavec[1], Ž. Budinšćak[1], I. Križanac[1], D. Škorić[2], M. Šeruga Musić[2]
[1]Institute for Plant Protection, Department for Diagnostics and Analytics, Zagreb, Croatia
[2]University of Zagreb- Faculty of Science, Department of Biology, Zagreb, Croatia

Backgrounds

Phytoplasmas are plant-pathogenic bacteria with a complex two-kingdom-host life cycle involving plants and insect vectors. Flavescence dorée (FD) phytoplasma is a quarantine pathogen associated with severe and epidemic grapevine yellows disease that represents great threat for grapevine cultivation in Europe.

Objectives

From the first report in Croatia (2009) for restricted continental area, FD phytoplasma has been detected in other winegrowing regions of the country with clear epidemic trend of the disease spread. The main objective of this study was to trace FD phytoplasma emergence, to study the prevalence and distribution of FD strains and determine their epidemiological significance by multilocus sequence typing (MLST).

Methods

In the 6-year period over 600 samples of grapevine, other wild/reservoir plants, *Scaphoideus titanus* and other potential vectors from different regions of Croatia were collected countrywide and analysed. Triplex real-time PCR was performed for detection together with PCR/RFLP of 16S rDNA. Detected FD phytoplasma isolates were characterised by MLST of *secY*, *map* and *uvrB-degV* genes.

Conclusions

FD-related phytoplasmas were found for the first time in alder, invasive tree species *Ailanthus altissima* and leafhopper *Phlogotettix cyclops*. Phylogenetic analyses of FD phytoplasma isolates revealed the existence of 3 mapFD strain clusters: mapFD1, mapFD2 and mapFD3, with the mapFD2 being prevalent. In addition, 7 *uvr*B-*deg*V and at least 5 *sec*Y genotypes have been detected. Finding of all 3 mapFD clusters and different distribution of genotypes based on MLST suggests separate routes of the disease introduction and propagation origins in Croatia. Tentative role of *P.cyclops* in transmission of FD phytoplasma is also indicated.

FEMS7-2681
Pathogens / Pathogenicity - Part III

COMPARISON OF TIGECYCLINE SUSCEPTIBILITY TESTING METHODS FOR MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII

S. Popovic[1], N. Opavski[1], I. Gajic[1], V. Mijac[1], D. Kekic[1], S. Jovanovic[2], A. Smitran[3], M. Lopicic[4], L. Ranin[1]
[1]Institute of Microbiology and Immunology Medical Faculty- University of Belgrade, Department of Microbiology and Immunology, Belgrade, Serbia
[2]Clinical center of Serbia, Department of Microbiology-, Belgrade, Serbia
[3]Faculty of medicine- University of Banja Luka, Department of Microbiology and Immunology, Banja Luka, Bosnia - Herzegovina
[4]Insitute for public health, Department for microbiology, Podgorica, Montenegro

Backgrounds

Multidrug-resistant (MDR) *Acinetobacter baumannii* is increasing worldwide, raising the necessity of finding effective therapies.

Objectives

The aim of this study was to compare broth microdilution (BMD) method and E test for tygecycline susceptibility testing of MDR *A. baumannii*.

Methods

A total of 133 MDR isolates of *A. baumannii* collected from hospital's intensive care units from Serbia, Montenegro and Bosnia and Herzegovina in 2016 were studied. Bacterial identification was done by Vitek-2 (bioMérieux®), while the minimal inhibitory concentrations (MICs) of tygecycline were determined by BMD and E test (Liofilchem®). Interpretation to susceptible, intermediate or resistant category was performed according to Enterobacteriaceae breakpoints (no tigecycline breakpoints available for A. baumannii) from both EUCAST (≤1, 2, ≥4) and FDA (≤2, 4, ≥8).

Conclusions

The MIC_{50}/MIC_{90} for BMD and E test were 4/8mg/L and 0.5/4, respectively. MIC range for BMD and E test were 0.25–32mg/L and 0.25-12mg/L. Essential agreement (EA, MIC difference ≤1 double dilution step) for BMD and E test amounted to 88%. With EUCAST breakpoints, categorical agreement (CA) for MBD and E test was achieved in 38% of isolates. Major discordance (MD, false susceptibility/false resistance) and minor discordance (mD, false categorization involving intermediate results) were thereby observed in 10%/57% of isolates. With FDA breakpoints, CA for BMD and E test was achieved in 44%, while MD and mD were observed in 16% and 47% of isolates.Our study highlights the differences between BMD and E test, which sometimes may lead to the restriction of the available treatment options or inappropriate therapy.

FEMS7-0831
Pathogens / Pathogenicity - Part III

COMPARATIVE PREVALENCE OF STAPHYLOCOCCAL ENTEROTOXIN GENE IN METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS ISOLATED FROM DOG, HUMAN, AND ENVIRONMENT IN A VETERINARY HOSPITAL

N. Prapasarakul[1], *N. Phumthanakorn*[1]
[1]*Chulalongkorn university, Veterinary Microbiology, Bangkok, Thailand*

Backgrounds

Methicillin-resistant *S. pseudintermedius* (MRSP) isolated from dogs can occasionally colonizes on nasal carriage in human and persists in environment. The source of bacterial isolation may be a factor related to bacterial-host adaptation that leads to genetic variation encoding viability and function.

Objectives

To detect and compare staphylococcal enterotoxin (*se*) family genes in MRSP divided by lineage sequence types that isolated from dogs, human, and the environment in veterinary hospital.

Methods

A total of 93 MRSP were isolated from dogs (n = 43), human (n = 18) and in a veterinary hospital (n = 32) in Thailand during 2009-2014. The detection of 17 *se* and toxic shock syndrome toxin (*tst*) genes was carried out by the single and multiplex PCR. Multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were used as DNA fingerprinting platform. The representatives of MRSP belonging to ST45 (n= 18), ST181 (n=8) and ST112 (n=8) were further selected for the comparison of *se* gene profiles within these clone types.

Conclusions

MRSP from human apparently possessed the most variation genes (12/14; *sea, sec, seg, sei, sek, sel, sem, sen, seo, sep, seq* and *tst-1*) followed by dog MRSP (5/14; *sec, sel, sem, seq* and *tst-1*) and the veterinary hospital (3/14; *sec, seq* and *tst-1*), respectively. The clone types seemed not to relate to number and type of *se* family gene profile. In contrast, the source of isolation might have a linkage to virulent genes. This finding highlights a higher pathogenicity potential of human MRSP than in the other sources.

FEMS7-1288
Pathogens / Pathogenicity - Part III

MULTILOCUS SEQUENCE TYPING OF MYCOPLASMA AGALACTIAE TO STUDY ITS GENETIC VARIABILITY IN A CONTAGIOUS AGALACTIA ENDEMIC AREA

M. Prats-van der Ham[1], J. Tatay-Dualde[1], Á. Gómez-Martín[1], A. Paterna[1], J.C. Corrales[1], A. Sánchez[1], A. Contreras[1], C. de la Fe[1]
[1]University of Murcia, Animal Health, Murcia, Spain

Backgrounds

M. agalactiae (Ma) is the main causative agent of contagious agalactia (CA), a serious disease of small ruminants. In endemic areas, the lack of effective strategies against this disease highlights the need of using molecular typing tools, such as multilocus sequence typing (MLST), to obtain epidemiological information which allows tracing the source of disease outbreaks and understanding the genetic distribution and evolution of pathogens, favouring disease surveillance and control.

Objectives

The aim of the present work was to assess the genetic variability of Ma isolates from a CA-endemic area considering all the epidemiological determinants associated to this disease, such as small ruminant host (sheep and goats) and separate geographical areas.

Methods

Sixty-three Ma isolates from 39 different ovine and caprine herds, retrieved between 2009 and 2016, were subjected to a previously described MLST scheme, which is based on the study of housekeeping genes *dnaA*, *gltX*, *gyrB*, *metS* and *tufA*. Distinct allelic numbers and subsequent sequence types (STs) were assigned according to the pubMLST database of Ma (http://pubmlst.org/magalactiae/), and STs were grouped in clonal complexes.

Conclusions

Although Ma is genetically more stable than other CA-causing mycoplasmas, genetic diversity appeared, mainly between caprine isolates. This variability could be explained by the coexistence of different CA-causing mycoplasma species infecting the same host, especially considering the possibility of horizontal gene transfer. The existence of different genetic profiles of Ma within a CA-endemic area affects the design of effective preventive and control strategies against this disease.

Acknowledgements
This study was funded by project AGL2013-44771-R awarded by the Spanish Ministry of Economy, government of Spain, and co-financed by FEDER funds.

FEMS7-1294
Pathogens / Pathogenicity - Part III

MOLECULAR CHARACTERIZATION OF MYCOPLASMA AGALACTIAE FIELD ISOLATES WITH ACQUIRED RESISTANCE TO TYLOSIN

M. Prats-van der Ham[1], J. Tatay-Dualde[1], Á. Gómez-Martín[1], A. Paterna[1], J.C. Corrales[1], A. Sánchez[1], A. Contreras[1], C. de la Fe[1]
[1]University of Murcia, Animal Health, Murcia, Spain

Backgrounds

Mycoplasma agalactiae (Ma) is the main causative agent of contagious agalactia (CA), a serious disease of small ruminants. Antimicrobials are the main strategy applied when clinical outbreaks of this disease occur. The development of effective therapeutic strategies depends upon an accurate understanding of the antibiotic resistance mechanisms. However, the specific molecular mechanisms of acquired resistance of Ma to 16-membered macrolides have not yet been elucidated.

Objectives

The aim of the present work was to determine the minimum inhibitory concentration (MIC) values of different field isolates of Ma to tylosin, and to investigate the association between the obtained MICs and mutations in the *23S rRNA* genes and ribosomal proteins L4 and L22 of the studied isolates.

Methods

Fifty Ma field isolates, retrieved between 2009 and 2016, were analysed. Their antimicrobial susceptibility to tylosin (Tyl) was tested by determining their MIC, following previously recommended guidelines for mycoplasmas. Subsequently, partial genome sequences of domains II and V of both *23S rRNA* genes and ribosomal encoding sequences *rplD* (L4) and *rplV* (L22) were studied.

Conclusions

Nearly 40% of the studied field isolates of Ma were resistant to Tyl, showing a remarkable decrease in their susceptibility in comparison to previous works performed on this mycoplasma species. This increase in MIC values was associated to the presence of mutations in ribosomal protein L22 (Ser89Leu and Gln90Lys/His) and in domain V of the *23S rRNA* genes (A2058G) (*Escherichia coli* numbering). This is the first report of the molecular mechanisms involved in macrolide resistance of Ma.

Acknowledgements
This study was funded by project AGL2013-44771-R awarded by the Spanish Ministry of Economy, government of Spain, and co-financed by FEDER funds.

FEMS7-1826
Pathogens / Pathogenicity - Part III

ISOLATION OF GASTROINTESTINAL YEAST STRAINS AS POTENTIAL PROBIOTICS

D. Prieto[1], *E. Roman*[1], *R. Alonso-Monge*[1], *J. Pla*[1]
[1]*Dept. Microbiología II- School of Pharmacy- Universidad Complutense de Madrid, Microbiología II, Madrid, Spain*

Backgrounds

Most *Candida albicans* systemic infections arise from endogenous origin. This microbe is present in the majority of the human population, colonizing the gut as a harmless commensal. Some bacteria and a few fungi species have been proposed as probiotic to reduce the candidiasis originated in the gastrointestinal tract; however, the mechanisms are still not clear and continuous administration is required. A stable colonization would be needed to improve the probiotic effect.

Objectives

Isolation and analysis of non-pathogenic yeast strains in a mouse model of gut colonization and test their potential as probiotics against *C. albicans*.

Methods

Mouse gut colonization by fungal species was established by the administration of an antibiotic combination in the drinking water. Fungal isolates were identified from the gut of C57B/6 mice and identified through 16S rDNA sequencing. The ability to colonize and to prevent *C. albicans* colonization was tested by CFUs analysis from stools. Nystatin sensitivity was also assessed *in vitro* and *in vivo*. This work was supported by the grant from the Ministry of Economy and Competitiveness PCIN-2014-052 (INFECT-ERA).

Conclusions

Cyberlindnera fabianii was identified as a frequently yeast isolate from mouse gut under antibiotic treatment. In this mouse model, this specie reaches a very high and stable fungal levels in the gut (around 10^8 cells/g) for a prolonged time (>6 weeks) and shows resistance to Nystatin treatment. Although *C. fabianii* does not prevent *C. albicans* colonization in this model, it can be considered a good candidate to design future strategies to its prevention.

FEMS7-1366
Pathogens / Pathogenicity - Part III

ITRACONAZOLE IN COMBINATION WITH A MONOCLONAL ANTIBODY SPECIFIC TO NEUTROPHIL REDUCES THE EXPRESSION OF GENES RELATED TO PULMONARY FIBROSIS IN AN EXPERIMENTAL MODEL OF PARACOCCIDIOIDOMYCOSIS

J.D. Puerta Arias[1], P.A. Pino Tamayo[2], J.C. Arango[1], L.M. Salazar Pelaez[3], A. Gonzalez[4]
[1]Corporación para Investigaciones Biológicas, Medical and Experimental Micology Group, Medellín, Colombia
[2]Weill Cornell Medical College, Microbiology and Immunology, New York, USA
[3]Universidad CES, Basic Sciences Group, Medellín, Colombia
[4]Universidad de Antioquia, Basic and Applied Microbiology Research Group MICROBA, Medellín, Colombia

Backgrounds

Itraconazole (ITC) is the drug of choice for treating paracoccidioidomycosis (PCM); nonetheless, most patients with the chronic form of PCM develop fibrosis, even after treatment. Recently, we observed that the depletion of neutrophils with a specific monoclonal antibody (anti-Ly6G) during the chronic phase of PCM was associated with a decrease in the fungal burden and the inflammatory response as well as with a reduction of fibrosis.

Objectives

To evaluate the effect of ITC in combination with the anti-Ly6G mAb using an experimental model of pulmonary PCM.

Methods

BALB/c male mice were challenged with *Paracoccidioides brasiliensis* yeasts, treated with the mAb and/or ITC at 4th week p.i. and then sacrificed at 12th week p.i. to assess neutrophil subpopulations, fungal load, expression of pro-inflammatory and pro-fibrotic genes, collagen levels, and pulmonary histopathological changes.

Conclusions

We observed that combination of mAb/ITC favored the control of infection and diminished the inflammatory response. Of note, such combined therapeutic strategy reduced synergistically the expression of IL-1β, IL-6, IL-17, TNF-α, TGF-β1, TGF-β3, GATA-3, RORc, Ahr, MMP-1α, MMP-8, MMP-15, TIMP-1 and TIMP-2 genes in comparison with those mice treated with the mAb or ITC alone. Interestingly, ITC induced an increase of type-II neutrophils, even in those mice treated with the antibody. These results indicate that combination anti-Ly6G mAb/ITC synergistically reduced the infection and pulmonary fibrosis through down-regulation of pro-inflammatory- and pro-fibrotic genes. This work emphasizes the importance of exploring new potential combination therapies to treat fungal infections.

FEMS7-1785
Pathogens / Pathogenicity - Part III

SPINASES – SECRETED SERINE PROTEASES FROM STAPHYLOCOCCUS PSEUDINTERMEDIUS

K. Pustelny[1], M. Wisniewska[2], P. Mak[3], J. Worwa[1], W. Rut[4], P. Golik[1], M. Drag[4], B. Wladyka[3], G. Dubin[1]
[1]Jagiellonian University, Department of Microbiology, Krakow, Poland
[2]Malopolska Centre of Biotechnology, Department of Structural Biology, Krakow, Poland
[3]Jagiellonian University, Department of Analytical Biochemistry, Krakow, Poland
[4]Wroclaw University of Technology, Faculty of Chemistry, Wroclaw, Poland

Backgrounds

Staphylococcus pseudintermedius, a member of *Staphylococcus* genus, is the most frequent bacterial pathogen isolated from canine clinical specimens. Staphylococci are known to produce a wide variety of exoproteins that promote host-pathogen interaction and eventually lead to infection. Since knowledge about *S.pseudintermedius* secretome is limited and almost all predictions concerning its role are mostly based on extrapolation to *S.aureus*, the pathogen calls for specific attention.

Objectives

This study presents a comprehensive analysis of recently identify *S.pseudintermedius* secreted proteases – Spinases, and aims at understanding of their role in pathogenesis.

Methods

The PCR analysis reveals the presence of eight ***spinases'*** genes (*spinaseA-H)* in all tested *S.pseudintermedius* isolates recovered from skin abscess lesions in dogs. The semi-quantitative RT-PCR demonstrates that Spinases are translated from individual gene transcripts controlled by independent promoters. Spinases' genes are mainly expressed during the transition into a stationary phase and a level of transcripts depends on culture condition. To conduct the biochemical and structural characteristic of SpinaseA, we produce and purify an active recombinant enzyme. The P4-P1 substrate specificity of the enzyme is next determined with a combinatorial library of synthetic peptide substrates. Crystallography and molecular modeling, were applied to explain the substrate specificity is explained at a molecular level.

Conclusions

Spinanes constitute a group of proteolytic enzyme with a potential role in staphylococcal infection. The proteases expression is tightly regulated in a response to environmental stimuli. The Spinase A reveals a restricted substrate specificity, similarly to staphylococcal V8 protease and epidermolytic toxins, well defined by arrangement of substrate binding pockets.

Acknowledgment

This work was financially supported by the grant from Jagiellonian University (KNOW K/DSC/003702).

FEMS7-3061
Pathogens / Pathogenicity - Part III

IDENTIFICATION OF THE SIDEROPHORE ACINETOBACTIN EXPORTER IN AEROMONAS SALMONICIDA SUBSP. SALMONICIDA

D. Rey Varela[1], M.C. Afonso Lages[1], M. Balado Dacosta[1], M.L. Lemos Ramos[1]
[1]Institute of Aquaculture, Microbiology and parasitology, Santiago de Compostela, Spain

Backgrounds

Aeromonas salmonicida subsp. *salmonicida* is the aetiological agent of furunculosis, a disease that affects a great variety of fish cultures worldwide. To obtain iron from its hosts, *A. salmocida* produces two siderophores: acinetobactin and amonabactin(s). The genes encoded the synthesis and transport of both siderophores have been previously described by our group. However the proteins involved in the secretion of these siderophores to the external medium are unknown.

Objectives

The main objective of this work was to identify and characterize the genes encoding the siderophores secretion function.

Methods

An *in silico* analysis of the genomes of different strains revealed the existence of 3 candidate genes to encode the corresponding efflux proteins: *asbH* and *asbJ*, which could encode the acinetobactin exporter, and *amoS* which could encode the amonabactins exporter. To test the function of these genes, we generated by allelic exchange single and combined mutants of all them, using a previously generated biosynthetic mutant for each siderophore. Using bioassays, under iron-restricted conditions, we tested the ability of each mutant to export the siderophores.

Conclusions

The results showed that only the mutants defective for *asbH* were unable to export acinetobactin to the medium, whereas the a*moS* mutant did not show any growth impairment in iron-deficiency conditions. In addition, the double mutant *asbH-amoS*, also kept the ability to secrete amonabactins to the medium. These results confirm our initial hypothesis that AsbH could be the acinetobactin exporter, but we still ignore the pathway followed by amonabactins to be secreted to the external medium.

FEMS7-3264
Pathogens / Pathogenicity - Part III

GENOMIC CHARACTERIZATION OF ENTEROAGGREGATIVE ESCHERICHIA COLI (EAEC) CLINICAL ISOLATES FROM CHILDREN IN AHVAZ

H. Helalat[1], S.E. Rezatofighi[1], M. Roayaei Ardakani[1]
[1]Shahid Chamran University of Ahvaz, Biology, Ahvaz, Iran

Backgrounds

Enteroaggregative *Escherichia coli* (EAEC) is identified as the emerging cause of pediatric diarrhea in developed and developing countries.

Objectives

Our study investigated the genetic characteristics of EAEC strains isolated from children with diarrhea in Ahvaz, Iran.

Methods

EAEC strains were identified using *agg*R primers which are specific for the pathotype. Thirty three EAEC strains were detected and examined for virulence genes of *aggA, aaF, aap, and pcvD by PCR.*

Conclusions

The *pcv*D was found in all of strains. The *agg*A gene was present in 24.24% of isolates and 87.9% of strains had the *aap* gene. However, the *aaf* gene was found in the any of strains. Combination of the genetic markers was different among the EAEC strains. However, the most prevalent combination was *pcv*D/*agg*A/*aap* that found in 24.24% of strains. Our data suggest that *pcvD and aggR* may serve as a valuable marker for the detection of EAEC pathotype and genetic heterogeneity is common in EAEC strains.

FEMS7-1801
Pathogens / Pathogenicity - Part III

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF PUCCINIA PORRI ON LEEK IN SERBIA

D. Ristic[1], I. Vucurovic[1], M. Stevanovic[1], S. Stosic[1], K. Gasic[1], S. Zivkovic[1]
[1]Plant Protection and Environment, Plant Disease, Belgrade, Serbia

Backgrounds

Leek rust, caused by *Puccinia porri* (Sow.) Winter is an economically important disease of the leek crop in Serbia. The bright orange urediniospores appear on young leaves and the disease spreads under warm damp conditions, resulting a reduction in plant growth and crop quality.

Objectives

Consequently, the objectives of this study were to identify the rust fungi on leek based on morphological and molecular characteristics.

Methods

Leaves samples infected with a rust fungus were collected from commercial leek crops (*Allium porrum*) in the locality Medveđa (Rasina district) during 2016. Small lesions on leaves with uredinia were present. Based on morphological characteristics and molecular diagnostics the pathogen was identified as *Puccinia porri.* The light brown urediniospores were ellipsoidal to obovoid and measured 24-32 × 20-25 µm. Morphological identification was confirmed by amplification and sequencing of the internal transcribed spacer (ITS) region. Total DNAs were extracted directly from fungal tissue with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and PCR amplification was performed with primers ITS1F/RUST1. Sequence analysis of ITS region revealed that representative isolate 22-16 (GenBank Accession No. KY492366) shared 100% identity with sequences of *P. porri* deposited in the GenBank.

Conclusions

Further characterization and research on diversity of rust fungi on *Allium* species in Serbia are needed. Increase of rust inoculum could lead to a higher incidence of the disease on leek and other *Allium* species and high yield looses.

This research was supported by the projects TR31018 funded by the Ministry of Education, Science and Technological Development, Republic of Serbia.

FEMS7-0215
Pathogens / Pathogenicity - Part III

IDENTIFICATION OF NEW INTESTINAL PATHOGENIC E. COLI ANTIGENS BY REVERSE VACCINOLOGY APPROACH

M. Rojas Lopez[1], *M. Pizza*[2], *M. Desvaux*[1], *R. Rosini*[2]
[1]*Institute Nationale pour la Recherche Agronomique, UR454 Microbiologie, Site de Theix, France*
[2]*GSK, Vaccines, Siena, Italy*

Backgrounds

The need for a vaccine development against Intestinal Pathogenic *Escherichia coli* (InPECs), lies in the increasing burden of the *E. coli*-diarrheal diseases; the emergence of hybrids and antibiotic resistance strains; and the heighten annual cost of the health care systems. Previous studies have shown the utility of the Reverse Vaccinology (RV) approach to identify new antigens as promising vaccine candidates in Extraintestinal Pathogenic *E. coli*.

Objectives

To identify novel antigens against InPECs

Methods

We applied the RV approach in two complete, annotated reference InPEC genomes: ETEC and EHEC. First, we selected CDSs mainly encoding extracellular and OMPs by predicting their cellular localization with the PSORT algorithm and further structures or protein features using the InterProScan server. To predict their expression in a transcriptional level, we went through RNA-seq reported experiments performed in different growth conditions and selected those expressed with >10 RPKM. To select antigens that have more than one target strain, we performed a gene distribution and variability analysis by BLAST among a panel of 47-*E. coli* complete genomes strains, using as cut-off criteria a query coverage of >90% and >80% of sequence identity. From this *in silico* analysis, we identified 31 potential antigens present in more than 5 pathogenic and fecal *E. coli* isolates, but not previously reported. The prospective antigens were cloned, expressed as recombinant proteins, and used to immunize mice to obtain specific sera. The specific sera were used in western blot analysis to detect the real expression of these proteins in total extracts preparations from standard laboratory conditions. The final selection panel includes 4 potential candidates

Conclusions

In conclusion, our study identified new potential vaccine candidates by their putative function, cellular exposure, expression, and genetic diversity in the different *E. coli* pathotypes, for the further development of a vaccine against InPECs strains infections, currently in immunological tests.

FEMS7-2207
Pathogens / Pathogenicity - Part III

PNEUMOCYSTIS CARINII INDUCES MUC5B HYPERSECRETION THROUGH STAT6/FOXA2 PATHWAY ACTIVATION IN IMMUNOCOMPETENT INDIVIDUALS.

D.A. Rojas[1], P. Iturra[1], A. Méndez[1], C. Ponce[1], R. Bustamante[1], S.L. Vargas[1]
[1]Facultad de Medicina- Universidad de Chile- ICBM, Programa Microbiología y Micología, Santiago, Chile

Backgrounds

Mucus hypersecretion is associated to respiratory diseases and is induced via several pathways such as STAT6/FoxA2 or EGFR. We have reported increased mucus associated to *Pneumocystis* infection in autopsied lungs of infants dying in the community. MUC5AC was tested as a marker of mucus because this protein is believed to be the most abundant mucin in airways. However, because *Pneumocystis* is likely the most frequent and consistent infection during early infancy, pathways and expression of other mucins such as MUC5B, essential in defense, need to be characterized.

Objectives

To study the role of MUC5B and STAT6/FoxA2 pathway during *Pneumocystis* primary infection in infants and immunocompetent *Pneumocystis*-infected rats.

Methods

Lungs from *Pneumocystis*-infected infants and rats were analyzed by qRT-PCR and western blot to evaluate mucins and STAT6/FoxA2 pathway members. STAT6/FoxA2 pathway was evaluated using Kaempferol on infected animals. Binding of FoxA2 to MUC5B promoter was evaluated by ChIP. Mucus hypersecretion was analyzed in fixed-airway sections.

Conclusions

MUC5B levels were elevated in *Pneumocystis*-infected compared to non-infected infants. The expression of MUC5AC and MUC5B was found increased in infected rats compared with non-infected animals. MUC5B mRNA and protein levels were higher than MUC5AC in infected animals suggesting MUC5B plays a role in defense against *Pneumocystis*. These increments were dependent on STAT6 pathway induction. Analysis of MUC5B promoter showed that FoxA2 occupancy decreased during infection in a STAT6 pathway-dependent manner. These results are showing that *Pneumocystis* primary infection induces mucus hypersecretion through STAT6/FoxA2 pathway suggesting a new mechanism and proposing a new pharmacological target for *Pneumocystis* infection.

FEMS7-1094
Pathogens / Pathogenicity - Part III

OVERPRODUCTION OF CYTOSOLIC CATALASE COUNTERBALANCES THE FUNGICIDAL ACTION OF DIFFERENT ANTIFUNGALS IN CANDIDA ALBICANS

E. Román[1], A.D. Prieto[1], E. Varea[1], R. Alonso-Monge[1], O. Zaragoza[2], J. Pla[1]
[1]Universidad Complutense de Madrid, Microbiología II, Madrid, Spain
[2]Instituto de Salud Carlos III, Micología, Majadahonda- Madrid, Spain

Backgrounds

Amphotericin B is a polyene type antifungal that interacts with fungal membranes leading to their loss of functionality and eventual cell death. The mechanism of action of this antifungal has been also shown to involve the production of reactive oxygen species (ROS). Mitogen activated kinases (MAPK) are involved in the response to ROS in the fungal pathogen *Candida albicans* and mutants defective in the *hog1* MAPK are sensitive to oxidants

Objectives

Determine the role that overproduction of cytosolic catalase (encoded by *CAT1*) has on the resistance of this fungus to antifungals and other drugs, morphogenesis and virulence

Methods

Strains overexpressing the *CAT1* under the control of the under the control of the tetracycline regulated system TET-ON/TET-OFF were constructed in a wild type and *hog1* background

Conclusions

Cells overexpressing *CAT1* are more resistant to certain oxidants such as hydrogen peroxide but not others, such as menadione. In addition, they were found to be more resistant to amphotericin B or ciclopirox olamine but not fluconazole. Overexpression of catalase also protected cells against mammalian phagocytic cells in both backgrounds. Virulence studies showed that *CAT1* overproduction was sufficient to suppress the avirulent phenotype of *hog1* mutants. Finally, we observed that MAPKs activation pattern under hydrogen peroxide, amphotericin B and fluconazole was diminished in *CAT1* overproducing cells

Our results indicate the important role for ROS in antifungal resistance and show that the production of catalase counterbalances the effects of certain antifungals, phagocyte-mediated killing and virulence in a systemic mouse model of candidiasis

FEMS7-3257
Pathogens / Pathogenicity - Part III

MICROSCOPE BIOINFORMATICS WORKFLOW TO PERFORM LARGE SCALE COMPARATIVE GENOMICS: APPLICATION TO ESCHERICHIA COLI BLOODSTREAM ISOLATES

G. Royer[1], D. Vallenet[2], V. De Lastours[3], J. Rollin[2], A. Lefort[3], O. Clermont[4], C. Laouénan[4], F. Mentré[4], E. Denamur[4], J.W. Decousser[5], C. Médigue[2], G. SEPTICOLI[6]
[1]CEA Genoscope / Université Paris Diderot / Assistance-Publique des Hôpitaux de Paris - Hôpital Henri Mondor, LABGeM / INSERM IAME UMR 1137 / Laboratoire de bactériologie, Evry, France
[2]CEA Genoscope, LABGeM, Evry, France
[3]Université Paris Diderot / Assistance-Publique des Hôpitaux de Paris - Hôpital Beaujon, INSERM IAME UMR 1137 / Service de Médecine Interne, Paris, France
[4]Université Paris Diderot, INSERM IAME UMR 1137, Paris, France
[5]Université Paris Diderot / Assistance-Publique des Hôpitaux de Paris - Hôpital Henri Mondor, INSERM IAME UMR 1137 / Laboratoire de bactériologie, Paris, France
[6]Université Paris Diderot / Assistance-Publique des Hôpitaux de Paris / CEA Genoscope, INSERM IAME UMR 1137 / APHP / LABGeM, Paris, France

Backgrounds

Bloodstream infection (BSI) is one of the most common infectious diseases worldwide, leading to important health expenditure. *Escherichia coli* is the major causative agent but also the most abundant species among facultative anaerobic bacteria in the human gut. To explain this paradox, many traits seem to be associated with pathogenicity including host factors, portal of entry, genetic and metabolic diversity of the strains. Furthermore, the worldwide epidemiology of *E. coli* BSI shows a dramatic shift since 2000 with the emergence of highly virulent and resistant clones, both from hospital and community settings. A large multicenter prospective study, “Septicoli”, has been launched by the IAME laboratory (Inserm-UMR1137) across 7 hospitals in Paris. Through 500 adults with *E. coli* BSI, accurate clinical data are collected and bacterial strains are isolated and sequenced (Illumina 2x150bp).

Objectives

We present here the bioinformatics workflow that is used to analyze *E. coli* genomes in order to find genetic determinants that may be associated to BSI severity.

Methods

For each strain, an extensive genome analysis is conducted using the MicroScope platform for comparative genomics and also: Mash pairwise comparison and phylogroup assignation, virulence (VFDB, VirulenceFinder) and antimicrobial resistance (CARD) gene prediction. Furthermore, a pangenome-based approach will be conducted focusing on strain metabolic capacities that could paly a role in strain pathogenicity

Conclusions

This bioinformatics analysis should help the interpretation of clinical data with the objective to identify new microbiological risk-factors for severe or fatal *E. coli* BSI.

FEMS7-3153
Pathogens / Pathogenicity - Part III

ANTIMICROBIAL ACTIVITY OF STREPTOCOCCUS MUTANS CULTURE FILTRATE AGAINST CANDIDA ALBICANS

L. Ruano de Oliveira Fugisaki[1], J. Diane dos Santos[1], M. Sa Alves[1], R. Previate Medina[2], A. Olavo Cardoso Jorge[1], D. Helena Siqueira Silva[2], J. Campos Junqueira[1]
[1]Universidade Estadual Paulista / UNESP, Departamento de Biociencias e Diagnostico Bucal, Sao Jose dos Campos, Brazil
[2]Universidade Estadual Paulista / UNESP, NuBBE – Nucleo de Biossintese- Bioensaios e Ecofisiologia de Produtos Naturais- Instituto de Quimic a, Araraquara, Brazil

Backgrounds

Streptococcus mutans, recognized as the main cause of dental caries in the world, can be found along with *Candida albicans* in the dental biofilm. Products of the secondary metabolism of the human microbiota are being increasingly investigated because of their direct impacts on the host and other microorganisms.

Objectives

Here, we evaluated the effects of the crude extract obtained from *S. mutans* culture filtrate on *in vitro* biofilm formation and morphogenesis of *C. albicans.*

Methods

Firstly, *S. mutans* was grown in brain-heart infusion broth for 24h at 37°C under 5% CO_2. After, the standardized suspension of *S. mutans* containing 10^7 cells/mL was inoculated in brain-heart infusion broth for 4 h at 37ºC under 5% CO_2, and then, the culture was centrifuged and the supernatant was filtered through a 0.22 µm membrane filter. Then, the crude extract was obtained by extraction with EtOAc (3 x 50% of culture filtrate volume each) and dried in rotatory evaporator. Next, the biofilms were formed on the bottom of a 96-well microtiter plate for 48 h and the number of colony-forming units (CFU) was determined. The *C. albicans* filamentation study was performed in 24-well plate and analyzed by light microscopy.

Conclusions

The results showed a statistically significant reduction in biofilm viable cells ($p < 0.05$) and significant inhibition of hyphae formation of *C. albicans* when in contact with 5 mg/mL of the crude extract. These findings suggest that *S. mutans* secretes subproducts capable to inhibit the biofilm formation and morphogenesis of *C. albicans*.

FEMS7-0983
Pathogens / Pathogenicity - Part III

CONSTRUCTION OF AN EFFECTORLESS CITROBACTER RODENTIUM STRAIN TO STUDY THE IN VIVO COMBINATORIAL CONTRIBUTION OF TYPE III SECRETION SYSTEM EFFECTORS

D. Ruano-Gallego[1], *L.Á. Fernández*[2], *G. Frankel*[1]
[1]*Centre for Molecular Bacteriology & Infection CMBI, Life Sciences, London, United Kingdom*
[2]*Centro Nacional de Biotecnología - CSIC, Biotecnología microbiana, Madrid, Spain*

Backgrounds

The attaching and effacing (A/E) pathogens enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) are major global causes of intestinal infections. Through the Type III Secretion System they inject virulence factors (effectors) into enterocytes, altering the homeostasis of the host. Since EPEC is a human-restricted pathogen and EHEC colonises only large farm animals apart from humans, the mouse-restricted pathogen *Citrobacter rodentium* has been used as a surrogate model to study A/E pathogens *in vivo*. *C. rodentium* infection provides useful insights to the contribution of individual effectors by single gene mutations, but this approach does not consider redundancy and dependency of the effectors.

Objectives

The aim of this project is to deconstruct the function of the effectors *in vivo* using the *C. rodentium* model.

Methods

To address this, we have generated a set of *C. rodentium* strains, with accumulating sequential genomic deletions of the effector genes, using a scarless mutation system that leaves no antibiotic resistance.

Conclusions

Through this method, we have generated an effectorless *C. rodentium* strain (CR-0) that lacks all known effector genes and is unable to colonise mucosal surfaces, but retains the functionality of the T3SS, as demonstrated by reinserting effector genes into their original loci. Using this approach, we expect to generate CR-0 expressing the minimal number of effectors needed for the colonisation of the gastrointestinal tract.
This study will provide a new level of understanding of diseases caused by A/E pathogens and the fundamental biology of bacterial infections of mucosal surfaces, with direct relevance to host responses to infection and the alteration of the intestinal microbiota.

FEMS7-1757
Pathogens / Pathogenicity - Part III

EVADING DECONTAMINATION STRATEGIES; GENETIC BASIS OF THE PERSISTENCE OF LISTERIA MONOCYTOGENES IN FOOD PROCESSING INDUSTRIES AND THE KNOCK-ON EFFECTS ON VIRULENCE

J.K. Rudkin[1], E.M. McCabe[2], C. Hill[1], C.G.M. Gahan[1]
[1]University College Cork, APC microbiome Institute. Microbiology Department., Cork, Ireland
[2]University College Cork, Microbiology Department, Cork, Ireland

Backgrounds

Listeria monocytogenes is an important foodborne pathogen which is present ubiquitously within the environment, representing a major source of food contamination. Methods to prevent contamination are often averted by *L. monocytogenes* due to high tolerance to many of the stresses which it is exposed to, persisting in the face of decontamination challenges. The genetic mechanisms underpinning this, and the evolutionary consequences of repeated stress exposure are not understood.

Objectives

This project aims to phenotypically characterise a large collection of strains isolated from food processing industries for tolerance to decontamination stresses. We shall assess the effects of repeated stress exposure on the evolution of strains to determine if high tolerance to stress conditions has any knock-on effects on virulence.

Methods

The effects of repeated stress exposure on survival, biofilm formation, and cellular invasion capacity shall be presented.

Conclusions

Both the stress response and the major virulence regulator (*prfA*) are co-regulated by the sigB operon, suggesting a link between stress tolerance and virulence in *L. monocytogenes*. We assessed the effect of environmental selection upon stress survival, and look at the linked effects on evolutionary adaptation and virulence of *L. monocytogenes* to answer the question; In trying to inhibit the growth of *L. monocytogenes* do we inadvertently drive pathogen evolution towards persistence, increased resistance and ultimately altered pathogenicity and virulence?

FEMS7-1304
Pathogens / Pathogenicity - Part III

TRANSCRIPTION ACTIVATOR LIKE EFFECTORS ARE INVOLVED IN THE HOST ADAPTATION OF XANTHOMONAS STRAINS RESPONSIBLE FOR COMMON BACTERIAL BLIGHT OF BEAN

M. Ruh[1], S. Bonneau[2], J. Foucher[2], A. Préveaux[2], L. Gagnevin[3], B. Szurek[4], L. Noël[5], M.A. Jacques[2], N.W.G. Chen[1]
[1]Agrocampus Ouest, Institut de Recherche en Horticulture et Semences IRHS, Beaucouzé Cedex, France
[2]INRA, Institut de Recherche en Horticulture et Semences IRHS, Beaucouzé Cedex, France
[3]CIRAD, Interactions Plantes Micro-organismes Environnement IPME, Montpellier, France
[4]IRD, Interactions Plantes Micro-organismes Environnement IPME, Montpellier, France
[5]CNRS, Laboratoire des Interactions Plantes Micro-organismes LIPM, Toulouse, France

Backgrounds

Transcription Activator Like Effectors (TALE) are type III effectors able to induce the expression of susceptibility genes to promote infection. To achieve this, TALE are able to get into the nucleus of the host cells, then specifically bind to the promoter region of targeted plant genes, resulting in the overexpression of these genes. Common bacterial blight of bean is caused by *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans* (*Xap-Xff*). *Xap-Xff* strains are spread across four phylogenetically distant lineages. Despite this genetic distance, these four genetic lineages have the ability to infect common bean. This leads us to hypothesize that these strains acquired common genetic determinants of adaptation to common bean, either by convergent evolution or by horizontal transfer.

Objectives

Our phylogenetic analyses indicate that *tal* genes have been transferred horizontally between distant *Xap-Xff* genetic lineages, suggesting that TALE would have been involved in the functional convergence of common bacterial blight agents.

Methods

In order to confirm this hypothesis, we undertook the functional validation of TALE shared by different *Xap-Xff* lineages. For this, we constructed *tal* deletion mutants and performed comparative analyses of transcripts from plants inoculated with bacterial strains containing TALE or not. Also, we performed phenotyping experiments including dynamics of bacterial population sizes and monitoring of symptom development by chlorophyll fluorescence Imaging.

Conclusions

Altogether, our results highlight how TALE impact common bean transcriptome and *Xap-Xff* pathogenicity, and confirm that TALE are involved in the adaptation of *Xap-Xff* to common bean.

FEMS7-1770
Pathogens / Pathogenicity - Part III

GENOMIC ANALYSIS OF PSEUDOMONAS AERUGINOSA INTRAPATIENT EVOLUTION IN NON-CF PATIENTS

P. Ruiz-Hueso[1], L. Sánchez-Busó[2], S. Harris[2], J. Parkhill[2], N. Tormo Palop[3], C. Gimeno Cardona[3], F. González-Candelas[1]
[1]FISABIO-Universidad de Valencia, Unidad Mixta Infección y Salud Pública, Valencia, Spain
[2]Wellcome Trust Sanger Institute, Pathogen Genomics, Cambridge CB10 1SA, United Kingdom
[3]Consorcio Hospital General Universitario Valencia, Servicio de Microbiología, Valencia, Spain

Backgrounds

Pseudomonas aeruginosa is a very dynamic bacterium, characterized by a highly variable genome. This trait, coupled with the complicated infections it causes, such as chronic lung infections in individuals with respiratory problems, and the high number of antibiotic resistance genes exchanged between species, make this bacterium a serious threat to public health. A better understanding of how these processes occur would help in the research of new treatments.

Objectives

To analyse genomic variations that could lead to adaptation in different body locations in non-cystic fibrosis patients.

Methods

We have analysed 92 samples taken at different time intervals and body locations from 15 patients. *P. aeruginosa* samples were isolated mainly from blood, urine, wounds and sputum. Pure cultures were sequenced with Illumina MiSeq 2x300bp. Genome assembly was performed with VelvetOptimiser, annotation of each genome with Prokka and preliminary analysis of the pangenome with Roary. Additionally, the reads were mapped to a reference strain with BWA. Putative recombinant regions were identified and removed with Gubbins and RAxML was used to reconstruct a phylogenetic tree.

Conclusions

Our results revealed a core of 1908 genes (present in >95% of the samples), from a total of 20818 detected genes. There is very large variability in the accessory genome as expected from the range of times and multiple sources sampled. Nevertheless, 80% of the samples belonged to ST244, a global *P. aeruginosa* clone which is associated with VIM-2 carbapenemase. Possible co-infection or re-infection with different clones was detected in 5 patients.

FEMS7-2046
Pathogens / Pathogenicity - Part III

BACTERIAL-DERIVED CELL-PENETRATING PEPTIDES DELIVER GENTAMICIN TO KILL INTRACELLULAR PATHOGENS

C. Rüter[1], M. Gomarasca[1], T. F.C. Martins[1], L. Greune[1], P. Hardwidge[2], M.A. Schmidt[1]
[1]Center for Molecular Biology of Inflammation, Infectiology, Münster, Germany
[2]College of Veterinary Medicine, Kansas State University, Manhattan, USA

Backgrounds

Commonly used antimicrobials show poor cellular uptake and often have limited access to intracellular targets, resulting in low antimicrobial activity against intracellular pathogens. An efficient delivery system to transport these drugs to the intracellular site of action is needed. Cell-penetrating peptides (CPPs) mediate the internalization of biologically active molecules into the cytoplasm.

Objectives

Here we characterized two CPPs, α1H and α2H, derived from the *Y. enterocolitica* YopM effector protein. These CPPs, as well as Tat, were used to deliver the antibiotic gentamicin to target intracellular bacteria.

Methods

The YopM-derived CPPs penetrated different endothelial and epithelial cells to the same extent as Tat. CPPs were covalently conjugated to gentamicin and CPP-gentamicin conjugates were used to target infected cells to kill multiple intracellular Gram-negative pathogenic bacteria, such as *E. coli* K1 RS218, *Salmonella enterica* serovar Thyphimurium, and *Shigella flexneri*.

Conclusions

Taken together, CPPs show great potential as delivery vehicles for antimicrobial agents and could contribute to the generation of new therapeutic tools to treat infectious diseases caused by intracellular pathogens.

FEMS7-3210
Pathogens / Pathogenicity - Part III

VIRULENCE STUDIES OF KLEBSIELLA PNEUMONIAE ISOLATED FROM NEONATAL NASOGASTRIC FEEDING TUBES

M. Saad[1], K. Dahmani[1], P. Ogrodzki[1], T. Osaili[2], J. Winter[1], S. Forsythe[1]
[1]Nottingham Trent University, School of Science and Technology, Nottingham, United Kingdom
[2]Jordan University of Science and Technology, Nutrition and Food Technology, Irbid, Jordan

Backgrounds

The rate of neonatal infections due to *Enterobacteriaceae* has increased in neonatal intensive care units, particularly in those born with low birth weights (< 2000g) and fed via nasogastric tubes.

Objectives

The aim of this study was to evaluate the potential virulence of *Klebsiella pneumoniae* isolated from neonatal nasogastric enteral feeding tubes (NEFT) from two Jordanian hospitals.

Methods

Seventy-six *K. pneumoniae* strains were isolated from NEFT, from May to Dec 2011. The isolates were genotyped using pulsed-field gel electrophoresis with *XbaI* and *SpeI* restriction digestion. Potential pathogenicity and biofilm-associated traits were determined using specific PCR probes, genome analysis, and *in vitro* tissue culture assays.
The *K. pneumoniae* strains were genotyped as sequence types (ST)111, 247 and 526. These isolates clustered into five pulsotypes (Kp 1-5) according to *XbaI,* which were confirmed using *SpeI* as the second restriction enzyme. *K. pneumoniae* Kp1 ST111 strains (n = 76) were obtained from 21 neonates, of 5 to 39 days old, from two hospitals. *K. pneumoniae* ST111 strains 1681 and 1725 exhibited various virulence traits associated with neonatal sepsis and extracellular matrix formation. The majority of isolates showed the ability to attach to and invade human bladder carcinoma cells(T24). Antibiogram analysis revealed that most strains were resistant to 3rdgeneration cephalosporins and produced ESBLs, whereas high susceptibility to ciprofloxacin was observed.

Conclusions

K. pneumoniae strains exhibited features that allow them to invade and survive within certain eukaryotic cells, and survive on feeding tubes. Therefore, NEFT are an important risk factor to consider with respect to neonatal infections.

FEMS7-2246
Pathogens / Pathogenicity - Part III

THE TRANSCRIPTION FACTOR ARCA MODULATES SALMONELLA TYPHIMURIUM RESPONSE TO THE CONDITIONS FOUND INSIDE NEUTROPHILS

C. Pardo-Esté[1], C. Cabezas[1], C. Aguirre[1], A. Briones[1], V. Cuevas[1], C. Salinas[1], M.S. Baquedano[1], J. Castro-Severyn[1], A. Hidalgo[2], M.C. Opazo[3], C.A. Riedel[3], C.P. Saavedra[1]
[1]Universidad Andres Bello, Molecular Microbiology Laboratory - Faculty of Biological Sciences, santiago, Chile
[2]Universidad Andres Bello,
Laboratory of Molecular and Antimicrobial Pathogenesis - Faculty of Medicine, santiago, Chile
[3]Universidad Andres Bello,
Millennium Institute of Immunology and Immunotherapy - Faculty of Biological Sciences, santiago, Chile

Backgrounds

Our study model *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) is a Gram-negative and intracellular pathogen that is able to avoid numerous defense mechanisms from the host´s immune response, here we focus on the phagocytes from the innate immune response like neutrophils. Inside these phagocytic cells, it faces several different kinds of stress; among the most relevant we found that highly toxic Reactive Oxygen Species (ROS), which inside neutrophils is mainly hypochlorous acid due to the myeloperoxidase action. In this context we have found that the ArcAB two-component system is also involved in ROS resistance to both hydrogen peroxide and hypochlorous acid.

Objectives

We proposed to determine the role of ArcA on the bacteria capacity to overcome the conditions inside the neutrophils.

Methods

To achieve this goal we set out to measure the transcriptional levels of relevant genes such as *sipC*, *sifA*, *sptP*, *sseI, cadB, pagC* and *manY* that are known to the up regulated during infection and in the presence of HOCl, as well as damage indicators such as lipid peroxidation and protein carbonylation in *S.* Typhimurium 14028s and *S.* Typhimurium D*arcA* strains harvested from infected murine neutrophils.

Conclusions

The ArcA regulon includes relevant genes for the bacterial survival inside neutrophils.

FEMS7-1317
Pathogens / Pathogenicity - Part III

MONOCLONAL ANTIBODIES DIRECTED TOWARD ACTINOMYCES NAESLUNDII POLYSACCHARIDE

A. Saeed[1], M. Pasciak[1], J. Szkudlarek[1], M. Drab[1], A. Gamian[1]
[1]Institute of Immunology and Experimental Therapy - Polish Academy of Sciences, Department of Immunology of Infectious Disease, Wroclaw, Poland

Backgrounds

Actinomycosis is a rare chronic disease caused by *Actinomyces* spp which are normal commensal flora of the oral cavity, gastrointestinal and urogenital tracts. In some cases this commensal flora becomes pathogenic when the mucosa barrier is breached.

Objectives

The goal of the study was to isolate and resolve the composition of polysaccharide antigens extracted from *Actinomyces naeslundii* to generate monoclonal antibodies reactive with them.

Methods

***Actinomyces naeslundii* were grown on liquid thioglycolate medium at 37 ºC for 120 hours in anaerobic atmosphere. Exopolysaccharide antigens were extracted by using trichloroacetic acid. The extracts were further purified by ion-exchange chromatography (DEAE Sephadex A25) and gel filtration (Toyopearal HW 55 S). The composition of pure exopolysaccharide was determined by gas-liquid chromatography–mass spectrometry of sugar alditol acetates. Monoclonal antibodies were produced by the hybridoma technique with whole cell mass immunization. The evaluation of specificity of monoclonal antibodies was carried out by ELISA method.**

Scanning electron microscopy showed rod-shaped bacteria, some of which had clubbed ends. Exopolysaccharide isolated from *A. naeslundii* consists mainly of glucose and traces of ribose and mannose. Two hybridomas named, mAbs 6 and mAbs 88, producing mAbs against exopolysaccharide antigen were selected. Both mAbs were of IgM class. The ELISA test allowed to detect cross reactivity of these monoclonal antibodies with different *Actinomyces* spp antigens, probably as a consequence of the common epitope present on the cell wall.

Conclusions

These preliminary results show *Actinomyces naeslundii* as rod-shaped bacteria with expolysaccharide antigen coat common among other Actinomycetes. We progress with the experimental work aimed to characterize the epitope detected by the monoclonal antibodies mAbs 6 and mAbs 88.

FEMS7-2937
Pathogens / Pathogenicity - Part III

IMMUNE EVASION MEDIATED BY A PSEUDOMONAS AERUGINOSA TIR DOMAIN-CONTAINING EFFECTOR

P. Imbert[1], A. Louche[1], J.B. Luizet[1], S. Bigot[1], S. Salcedo[1]
[1]CNRS UMR5086, Molecular Microbiology and Structural Biochemistry, Lyon, France

Backgrounds

Bacterial pathogens have developed numerous strategies to subvert the innate immune system to establish an infection. Several bacterial pathogens take advantage of TIR domain-containing proteins for blocking Toll-like receptor (TLR) signalling but the mechanisms that enable these proteins to interfere with receptor proximal events are still poorly characterized.

Objectives

We focused on a previously uncharacterized TIR domain-containing protein of the multi-drug resistant pathogen *Pseudomonas aeruginosa* PA7, that we called PumA. We aimed at identifying its cellular targets and characterize its role during infection.

Methods

By combining microbial genetics with cell biology and biochemistry approaches we have found PumA inhibits NFκB translocation into the nucleus during infection of A549 cells, a property transferable to non-PumA strain PA14. PumA directly interacts with the TLR adaptors MyD88 and TIRAP. Consistently, high-resolution microscopy imaging shows ectopically-expressed PumA co-localizing with both adaptors. More interestingly, PumA was found to directly interact with the ubiquitin-associated protein 1 (UBAP1), an important modulator of cytokine receptor sorting. Endogenous co-immunoprecipitation assays confirmed that PumA is associated with the endosomal-sorting complex required for transport I (ESCRT-I) of which UBAP1 is a major constituent.

Conclusions

By targeting UBAP1 and TLR adaptors via its TIR domain, PumA enables *P. aeruginosa* PA7 modulation of both cytokine and TLR signalling pathways, highlighting a novel strategy for bacterial innate immune evasion.

FEMS7-2172
Pathogens / Pathogenicity - Part III

WADD, A NEW GLYCOSYLTRANSFERASE ACTING ON BRUCELLA LIPOPOLYSACCHARIDE CORE SYNTHESIS, ITS INTERACTION WITH INNATE IMMUNE SYSTEM AND VIRULENCE

M. Salvador[1], *I. Moriyón*[1], *M. Iriarte*[1], *R. Conde-Álvarez*[1]
[1]*Universidad de Navarra, Microbiology and Parasitology, Pamplona, Spain*

Backgrounds

Brucellosis is a zoonotic disease caused by *Brucella*. The lipopolysaccharide (LPS) of *Brucella* plays a major role in virulence as impairs normal recognition by the innate immune system, and delays the immune response. The LPS core is involved in the resistance to complement and polycationic peptides. Mutants in glycosyltransferases involved in its synthesis are attenuated and good vaccine candidates against brucellosis. The chemical structure of the *Brucella* LPS core suggests that, in addition to the already identified WadB and WadC, other glycosyltransferases should also be implicated in its biosynthesis.

Objectives

Identification of new genes encoding glycosyltransferases involved in synthesis of *Brucella* LPS core and analysis of their role in virulence.

Methods

We constructed mutants in 7 not yet identified ORFs putatively encoding core glycosyltransferases in *B. abortus*. We analysed their LPS structure, sensitivity to different components of innate immune system and virulence.

Conclusions

All mutants kept the O-chain in their LPS. Interestingly, mutant in ORF BAB1_0953 (named wadD) lost reactivity against the antibodies that recognize the core section. This suggest that WadD is a new glycosyltransferase adding one or more sugars to the core ramification of *Brucella* LPS that is not linked to the O-chain. WadD mutants were more sensitive than the parental strain to components of the innate immune system. *In vivo* studies suggest that WadD plays a role in chronic stages of infection. This opens new perspectives for the design of new *Brucella* vaccines since it is known that mutants in the core branch protect against brucellosis.

FEMS7-1082
Pathogens / Pathogenicity - Part III

UNIQUE PERITONEUM MICROBIOME IN END-STAGE RENAL DISEASE PATIENTS AND THE INFLUENCE OF PERITONEAL DIALYSIS

L. Simões-Silva[1], M. Pestana[2], I.J. Soares-Silva[3], B. Sampaio-Maia[4]
[1]University of Porto, I3S-Instituto de Investigação e Inovação em Saúde, Porto, Portugal
[2]University of Porto, I3S-Instituto de Investigação e Inovação em Saúde- Faculdade de Medicina, Porto, Portugal
[3]University of Porto, I3S-
Instituto de Investigação e Inovação em Saúde- Centre of Biological Engineering, Porto, Portugal
[4]University of Porto, Faculdade de Medicina Dentária- I3S-
Instituto de Investigação e Inovação em Saúde- INEB-Instituto de Engenharia Biomédica, Porto, Portugal

Backgrounds

Factors influencing the occurrence of peritoneal dialysis (PD) related infections, namely peritonitis and catheter exit-site infections, are still far from being fully understood. Presently there is no data regarding the existence of a microbiome in the peritoneum.

Objectives

To understand if the peritoneum of end-stage renal disease (ESRD) patients harbours a unique microbiome and determine the influence of PD catheter insertion and PD technique in the peritoneal microbiome.

Methods

Peritoneal tissue from ESRD patients with intact peritoneal cavity (ESRD-nonPD, n=10) and ESRD in PD program with PD catheter (ESRD-PD, n=10) were collected under sterile conditions. Metagenomic approach included PCR amplification of V3-V4 region of 16S ribosomal gene using MiSeq Illumina® technology.

Conclusions

The peritoneum tissue of ESRD patients presents a unique microbiome dominated by the phyla Proteobacteria, Firmicutes and Actinobacteria. The catheter insertion for PD therapy may induce slight changes in the microbiome composition revealed by a non-statistically significant decrease in species richness after PD catheter insertion (α-diversity, $p = 0.056$) as well as a decrease in the prevalence of Lactobacillaceae, Peptococcaceae and Carnobacteriaceae families and an increase in the number of reads in Pseudomonadaceae, Bradyrhizobiaceae and Xanthomonedaceae families in the peritoneum tissue. Also, LEfSe analysis showed enrichment of *Halomonas* and *Mesorhizobium* and decrease in *Lactobacillus*, *Peptococcus* and *Facklamia* in ESRD with catheter insertion.

FEMS7-0893
Pathogens / Pathogenicity - Part III

EVOLUTION OF POXA-48-MEDIATED CARBAPENEM RESISTANCE IN A HOSPITAL SETTING

J. de la Fuente[1], *J. Rodríguez-Beltrán*[1], *R. León-Sampedro*[1], *M. Hernández*[1], *R. Cantón*[1], *Á. San Millán*[1]
[1]*Ramon y Cajal Institute for Health Research, Microbiology, Madrid, Spain*

Backgrounds

Plasmids mediate the horizontal transmission of genetic information between bacteria by conjugation, playing a pivotal role in the evolution of antibiotic resistance in pathogenic bacteria. Some of these plasmid-bacterium associations become particularly successful, creating superbugs that spread uncontrollably in clinical settings. The rise of these clones is mainly constricted because plasmids entail a fitness cost when they arrive in a new bacterial host. This cost can be subsequently alleviated through compensatory adaptation during plasmid-bacterium coevolution. Despite the importance of this cost-compensation dynamic in the evolution of plasmid-mediated antibiotic resistance, it remains completely unexplored in clinical contexts.

Objectives

In this work we analyse the spread and evolution of enterobacteria clones carrying the carbapenemase-coding plasmid pOXA-48 in the hospital Ramon y Cajal in Madrid.

Methods

We used molecular epidemiology and genome sequencing to characterized the inter-patient spread of enterobacteria clones carrying pOXA-48 over a two-year period in four different wards in the hospital. Moreover, we tracked the events of conjugation of pOXA-48 in the gut of hospitalized patients and analysed the fitness effects produced by the plasmid and the subsequent intra-patient evolution of plasmid carrying clones.

Conclusions

Klebsiella pneumoniae drives the dissemination of pOXA-48 among patients in the hospital. Once *K. pneumoniae*/pOXA-48 colonises a new patient pOXA-48 spreads horizontally towards other resident enterobacteria in the gut microbiome. The fitness effects of the plasmid in the new bacterial hosts determine the fate of these associations in the bacterial community.

FEMS7-0728
Pathogens / Pathogenicity - Part III

QUANTITATIVE ANALYSIS OF BISTABILITY IN SALMONELLA INVASION: A RE-INTERPRETATION

M.A. Sánchez-Romero[1], *J. Casadesús*[2]
[1]*University of Seville, Genetics, Sevilla, Spain*
[2]*University of Seville, Genetics, Seville, Spain*

Backgrounds

Salmonella enterica pathogenicity island 1 (SPI-1) is a gene cluster that encodes a type 3 secretion apparatus and effectors involved in invasion of human epithelial cells. A well known trait of SPI-1 is bistable expression, which generates SPI-1 ON and SPI-1 OFF subpopulations. The biological significance of SPI-1 bistability has been adressed by previous, insightful studies. Bistability has been viewed, for instance, as a division of labour involving self-destructive altruism by the SPI-1 OFF subpopulation (Ackermann et al. *Nature* 454, 987-90, 2008). Another study, however, has envisaged that the SPI-1 OFF subpopulation might benefit from inflammation triggered by the SPI-1 subpopulation (Stecher et al. *PLOS Biology* 5:2177-89, 2007).

Objectives

In this communication we describe an additional, unsuspected feature of SPI-1 bistability.

Methods

To do this, we have used a wide array of single cell assays to study the phenotypic heterogeneity during Salmonella virulence.

Conclusions

We show that relatively pure SPI-1 ON and OFF subpopulations obtained by bacterial cell sorting are observed non invasive, indicating that both subpopulations play an essential role in invasion. When individual Salmonellae are visualized inside epithelial cells, the intracellular bacterial populations are made of ON and OFF cells. These observations propose that the OFF subpopulation is able to invade, and can benefit from the activity of the invasion machinery of the ON cells. In support of this view, we also show that a small number of ON cells is sufficient for epithelial cells invasion, suggesting an uneven distribution of labor between ON and OFF cells.

FEMS7-1401
Pathogens / Pathogenicity - Part III

GENOME-WIDE IDENTIFICATION OF GENES REQUIRED FOR THE INTRACELLULAR SURVIVAL OF SALMONELLA TYPHIMURIUM IN THE AMOEBA DICTYOSTELIUM DISCOIDEUM

B. Labra[1], A. Sabag[1], C. Valenzuela[1], Í. Urrutia[1], S. Riquelme[1], S. Porwollik[2], M. McClelland[2], S.A. Álvarez[1], C.A. Santiviago[1]
[1]Universidad de Chile,
Departamento de Bioquímica y Biología Molecular- Facultad de Ciencias Químicas y Farmacéuticas, Santiago, Chile
[2]University of California, Department of Microbiology and Molecular Genetics- School of Medicine, Irvine, USA

Backgrounds

Salmonella spends a substantial part of its life cycle in the environment living in close contact with predatory organisms that feed on bacteria, such as protozoa. Recently, we described that *S.* Typhimurium can survive intracellularly in the social amoeba *Dictyostelium discoideum*. However, no in-depth study has been conducted to elucidate the molecular mechanisms used by *Salmonella* to survive within Dictyostelia.

Objectives

In this work, we performed a high-throughput analysis of mutants under selection to identify genes that contribute to *S.* Typhimurium survival in *D. discoideum*.

Methods

We infected *D. discoideum* AX4 with a transposon library containing ~200,000 mutants of *S.* Typhimurium 14028s. After co-incubation for 1 h, extracellular bacteria were removed and intracellular bacteria were recovered immediately (t0) and after 6 h of infection (t6). Total DNA from recovered bacteria was amplified across specific barcodes present in the transposon and sequenced to measure the relative amount of each transposon in the population of bacteria at t0 and t6.

Conclusions

Analysis of the sequencing data identified mutants in 185 genes that were under negative selection after 6 h of infection. These genes encoded products whose functions included lipopolysaccharide synthesis and modification (*wbaPKMNU, wbaICD*, *waaK*, *waaL*, wzz_{fepE}, *eptA*, *pmrAB*, *pmrD*, *arnEF*), metabolism (*aroA*, *aroC*, *gnd*, *fabF*, *pgpA*), and transport (*acrAB*, *mgtCB*, *trkD*), among others. Predictions from our high-throughput screening are being confirmed for a selected group of genes using competition assays between individual single-gene deletion mutants and the wild-type strain.

Supported by FONDECYT grants 1140754 and 1171844, and CONICYT fellowships 22140758, 21140615 and 21150005.

FEMS7-2045
Pathogens / Pathogenicity - Part III

CHEMOTAXONOMIC AND MOLECULAR CARACTERIZATION OF TENACIBACULUM MARITIMUM

B. S. Torres[1], N. Buján[1], A. E. Toranzo[1], B. Magariños[1]
[1]CIBUS- Facultad de Biología & Instituto de Acuicultura, Microbioloxía e Parasitoloxía- Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Backgrounds

Tenacibaculum maritimum, a Gram negative and filamentous bacterium, has been described as the etiological agent of tenacibaculosis in marine fish. Until now, tenacibaculosis is one of the most threatening bacterial infections limiting the culture of many species of commercial value in distinct geographical areas of the world.
At serological and molecular level, previous studies revealed the existence of intraspecific variability within this pathogen. In the last years, new outbreaks of tenacibaculosis occurred in different fish species which makes necessary in deep studies of this pathogen.

Objectives

The objective of this work is to study the existence of genetic and chemotaxonomic variability among different strains of *T. maritimum* isolated between 2007-2016 from several marine fishes with the aim to determine a possible association between genogroups and origin or serotype.

Methods

A total of 20 strains of *T. maritimum* were used in this work. All strains were identified by 16S rRNA gene sequencing and specific PCR. The molecular analysis was performed using ERIC, REP and RAPD PCR methodologies and the fatty acid composition was determined with the Sherlock Microbial Identification System.

Conclusions

The molecular results confirmed the existence of genetic variability within *T. maritimum* strains but not associations with the host and/or serogroups were found. With regard to the analysis of the fatty acid profiles, a low variability in their composition was observed among the strains analysed.

FEMS7-2254
Pathogens / Pathogenicity - Part III

SEARCH FOR POLYMORPHISMS IN THE ESPB AND ESPD PROTEINS AND THEIR EFFECT ON THE ADHERENCE OF ATYPICAL ENTEROPATHOGENIC ESCHERICHIA COLI TO HELA CELLS

F. Fernandes dos Santos[1], D. Yamamoto[1], R. Tavanelli Hernandes[2], R.M. Fontes Piazza[3], W. Pereira Elias[3], T. Aparecida Tardelli Gomes[1]
[1]UNIFESP - Universidade Federal de São Paulo, Departamento de Microbiologia- Imunologia e Parasitologia, São Paulo, Brazil
[2]UNESP - Universidade Estadual Paulista, Departamento de Microbiologia e Imunologia, Botucatu, Brazil
[3]Instituto Butantan, Laboratório de Bacteriologia, São Paulo, Brazil

Backgrounds

Intimin (encoded by *eae*) is the main adhesin of atypical enteropathogenic *Escherichia coli* (aEPEC). However, we have previously shown that an *eae* mutant of the aEPEC 1551-2 strain maintains its adherence efficiency by virtue of EspB and EspD, the components of a Type Three-Secretion System (T3SS) translocon.

Objectives

This study aimed to investigate whether polymorphisms in EspB and EspD could result in differences in the aEPEC efficiency to adhere to epithelial cells.

Methods

SDS-PAGE and Immunoblotting were used to determine the sizes of EspB and EspD of all eight strains studied. Intimin mutants were constructed from all strains, two of which were tested regarding any modification in their adherence efficiency on HeLa cells. The *espB* and *espD* genes were sequenced, translated into protein sequences and compared to those of the 1551-2 and EPEC prototype E2348/69 strains, using ExPasy and Clustal Omega. EspB and EspD molecular weights differed among the eight strains. Of the two mutants tested for adherence, one became non-adherent (BA4095), while the other (2012-1) remained adherent. Subsequently, the later mutant was used to generate an *eae*/*escN* (encoding the T3SS-ATPase) double mutant that was non-adherent, thus suggesting that its T3SS-translocon contributed to its adherence. The analyses of the EspD transmembrane domains have shown three identical aminoacids between the non-adherent *eae* mutant and the tEPEC strain, which were different from those present in the adherent 2012-1 and 1551-2 mutant strains.

Conclusions

Our preliminary analyses suggested that specific polymorphisms could contribute to the efficient adherence of aEPEC intimin mutants.

FEMS7-0248
Pathogens / Pathogenicity - Part III

STRUCTURE-FUNCTION RELATIONSHIP IN BACTERIAL ADHESINS

A. Saragliadis[1], *D. Linke*[1]
[1]*University of Oslo, Department of Biosciences, Oslo, Norway*

Backgrounds

Bacterial adhesion is a crucial step in bacterial pathogenesis. Adhesion prevents microbial clearance from host by mechanical forces and also contributes to successful colonization of cells and tissues. The specialized surface proteins that mediate bacterial adhesion are called adhesins. They recognize specific binding partners on surfaces of host cells, leading subsequently to attachment, biofilm formation and pathogenesis. For the optimal control of bacterial adhesion and biofilm formation it is important to pinpoint the means to prevent and inhibit the adhesion and therefore the formation of biofilms.

Objectives

The aim of this project is to obtain a better understanding of individual adhesins and of their binding modes, and of their biogenesis. The ultimate goal of this research is the development of antimicrobial compounds, i.e. direct or indirect inhibitors of bacterial adhesion.

Methods

In order to identify novel antimicrobial compounds we are developing assays in our labs that will allow us to screen for molecules with the desired functionality. Subsequently we will transfer our developed assay to the High-Throughput Chemical Biology Screening Platform, located at the Biotechnology Center in Oslo. There, we are aiming to set up the necessary pilot screen that will lead us to a target based screen against a diversity library containing at least 28500 compounds.

Conclusions

As soon as we have identified lead compounds and after a verification process, the successful candidates will be used to expand on the understanding of adhesion structure and function.

FEMS7-3208
Pathogens / Pathogenicity - Part III

LISTERIA SENSU STRICTU SPECIFIC GENES ARE IMPORTANT FOR IN VIVO COLONIZATION OF LISTERIA MONOCYTOGENES IN A FOODBORNE MOUSE MODEL

J. Schardt[1], G. Jones[2], S. Müller-Herbst[1], S. D'Orazio[2], T. Fuchs[3]
[1]Zentralinstitut für Ernährungs- und Lebensmittelforschung, Mikrobiologie, München, Germany
[2]College of Medicine University of Kentucky, Microbiology- Immunology & Molecular Genetics, Lexington- Kentucky, USA
[3]Friedrich-Loeffler-Institut, Institut für molekulare Pathogenese, Jena, Germany

Backgrounds

Listeria monocytogenes is the causative agent of listeriosis. Consumption of contaminated foods may lead to colonization of the gastrointestinal tract and a subsequent systemic infection.

The genus *Listeria* consists of 17 species, among them the two pathogens *L. monocytogenes* and *L. ivanovii*. Together with four other species, they form the so called "*Listeria sensu strictu*" group. All its members have been found in feces and the gastrointestinal tract of mostly symptom-free animals. The species of the second group in the genus, the "*Listeria sensu lato*", are thought to be solely environmental bacteria without the ability for colonization.

Objectives

To identify novel colonization determinants of *L. monocytogenes*, we applied a genome comparison approach between the two groups, and identified a list of candidate genes conserved in the *Listeria sensu strictu* species of which two were analyzed in more detail.

Methods

A mutant of a gene-pair encoding an ABC-Transporter exhibited a defect in adhesion and invasion of Caco-2 cells. Using a novel mouse model of foodborne infections (Bou Ghanem *et al*., 2013), a reduced number of the mutant strain was observed in the tissue of the small intestine. A mutant of a gene involved in propanediol metabolism showed reduced persistence in the stool of infected mice, pointing to a role of propanediol as carbon and energy source.

Conclusions

Taken together, these data revealed the presence of yet unknown colonization genes of *L. monocytogenes*. Both factors are also found in non-pathogenic *Listeria* species of the *sensu strictu* group, suggesting their potential to interact with mammalian hosts.

FEMS7-1222
Pathogens / Pathogenicity - Part III

INFLUENCE OF BACTERIAL STATE ON CRONOBACTER SAKAZAKII VIRULENCE

K. Schauer[1], *J. Stecklum*[1], *E. Märtlbauer*[1]
[1]*Ludwig-Maximilians-Universität München, Lehrstuhl für Milchhygiene, Oberschleißheim, Germany*

Backgrounds

Cronobacter sakazakii are Gram-negative, facultative anaerobic bacteria of the family *Enterobacteriaceae* that have been implicated in rare but severe cases of illnesses predominantly in premature and newborne infants. This opportunistic pathogen has been isolated from clinical as well as from a range of food and environmental sources. The ability to form biofilm increases the persistence of *C. sakazakii* under different harsh environmental conditions and delays the entering of bacteria in a VBNC (***v****iable* ***b****ut* ***n****on-****c****ulturable*) state.

Objectives

Although progress has been made during the last years, there is still a lack of knowledge on the virulence-associated factors and processes involved during pathogenesis.

Methods

In order to investigate the importance of biofilm formation for *C. sakazakii* pathogenicity and to examine the virulence of VBNC cells, bacteria cells from stationary growth phase were desiccated under standardized conditions, resuscitated and analyzed in a gentamycin protection assay in Caco-2 cells.

Conclusions

The results showed that two bacterial populations with distinct functions are present in the biofilm: aggregated cells form and conserve the biofilm in contrast to planktonic cells which are more virulent and swarm to build new aggregates. VBNC cells generated from planktonic cells are also more virulent compared to the VBNCs from aggregates.

This work was supported by the Federal Ministry of Education and Research (BMBF) of Germany (Food Supply and Analysis [LEVERA], funding code 13N12611).

FEMS7-0246
Pathogens / Pathogenicity - Part III

ASSAY DEVELOPMENT: ADHESIN INTERACTION WITH IMPLANT MATERIALS

K. Schneider[1], D. Linke[1], H.J. Haugen[2]
[1]University of Oslo, Department of Biosciences, Oslo, Norway
[2]University of Oslo, Institute of Clinical Dentistry - Department of Biomaterials, Oslo, Norway

Backgrounds

Dental implants have been optimised with rough surfaces for bone integration; however, such surfaces also promote the unwanted adhesion of pathogens. This results in an inflammatory response (peri-implantitis) in 28-56 % of patients, causing the loss of supporting bone, and ultimately leads to implant failure.

The first step during bacterial infection is the adhesion to host cells or other surfaces, mediated by adhesins (cell surface proteins). Adhesins require suitable receptors or material surfaces to function. Consequently, bacterial adhesion to implant surfaces can be regulated in two ways; surface modification with antagonistic or supporting molecules, or by targeting the biogenesis of the adhesins.

Objectives

A bottom-up approach, starting with molecular biology studies of bacterial adhesion factors, is necessary, so that strategic implant surface modifications can be developed. We therefore develop assays that can mimic the host environment and that deliver time-resolved data regarding the performance of various implant surfaces.

Methods

We are creating a feedback loop between a materials-science and a molecular biology laboratory by testing wild type, knockouts and overexpressed adhesins. This improves both the surface modifications and the assays, and guarantees direct translation of fundamental research into application. Aggregatibacter actinomycetemcomitans (Aa) serves as model organism as it is associated with the destructive inflammatory process surrounding infected dental implants (peri-implantitis), which can also cause inflammation of the aortic valve of the heart (mortality rate: 18%).

Conclusions

We expect answers to adhesin expression pathways and adhesin function, enabling us to target particular cell-substrate interactions in our modifications of implant surfaces.

FEMS7-1322
Pathogens / Pathogenicity - Part III

THE BEAT-AMR CONSORTIUM: PARTNERSHIP AGAINST BIOFILM-ASSOCIATED EXPRESSION, ACQUISITION AND TRANSMISSION OF ANTIMICROBIAL RESISTANCE

F. Schreiber[1], H.J. Kunte[1], Q. Ren[2], M. Buhmann[2], H. van der Mei[3], H. Busscher[3], S. Faust[4], J. Webb[5]
[1]Federal Institute for Materials Research and Testing BAM, Materials and Environment, Berlin, Germany
[2]EMPA - Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biointerfaces, St. Gallen, Switzerland
[3]University Medical Center Groningen, Department of Biomedical Engineering, Groningen, The Netherlands
[4]University of Southampton, Pediatric Immunology and Infectious Diseases and NIHR Wellcome Trust Clinical Research Facility, Southampton, United Kingdom
[5]University of Southampton, Biological Sciences, Southampton, United Kingdom

Backgrounds

Here, we introduce the BEAT-AMR consortium, which is recommended for funding within the 3rd call of the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR).

Objectives

The aim of the consortium is to investigate fundamental mechanisms that shape antimicrobial resistance in biofilms in relation to the surface and then translate those findings into clinical practice. We thereby aim to generate clinical recommendations on the combinatorial use of biomaterials coated with antimicrobials and antibiotics that avoid the occurrence and transmission of nosocomial biofilm infections with bacteria insusceptible to antibiotics.

Methods

We established a Europe-wide network of experts in biofilm research, antimicrobial resistance, material sciences, and translational medicine that allows us to investigate those aspects in a coherent framework.

Conclusions

Biofilms are structured communities of bacteria found on surfaces that become embedded within a self-produced extracellular polymeric matrix. Bacteria living in biofilms can tolerate much higher antibiotic concentrations compared to planktonic bacteria and survive long enough to evolve antimicrobial resistance (AMR). They form persistent, hard-to-treat infections and exhibit an intrinsic biology that promotes the development and transmission of AMR. The goal of our consortium is to determine how bacteria adapt to antimicrobials during biofilm formation on surfaces coated with antimicrobials, how AMR mutations are acquired and evolve within mature biofilms, and how population dynamics within biofilms affect the transmission of AMR. Our team provides facilities and clinical research governance for experimental and translational medicine. Our synergy of laboratory, clinical and translational research across Europe will ensure the development of novel and successful interventions and therapeutic outcomes.

FEMS7-0823
Pathogens / Pathogenicity - Part III

PRODUCTION OF PORCINE CIRCOVIRUS NUCLEOCAPSID PROTEIN IN SACCHAROMYCES CEREVISIAE

S.H. Seo[1], K.M. Park[1], Y.J. Shim[2], C.S. Kim[2], S.S. Yoo[2], E.S. Choi[1]
[1]Korea Research Institute of Bioscience & Biotechnology, Bio Process Engineering Center, Cheongju-si, Republic of Korea
[2]ChoongAng Vaccine Laboratories Co.- Ltd., Manufacture & Research, Daejeon, Republic of Korea

Backgrounds

Porcine circoviruses are associated with postweaning multisystemic wasting syndrome (PMWS). The nucleocapsid protein of porcine circovirus type 2 (PCV2) encoded by ORF2 is a promising subunit vaccine against porcine circovirus. Recombinant production of PCV2 ORF2 was studied in *Saccharomyces cerevisiae*.

Objectives

To develop a high-efficacy subunit vaccine of porcine circovirus using yeast.

Methods

For intracellular expression of ORF2, the gene was chemically synthesized according to yeast codon preference, was placed under GAL10 promoter and the resulting plasmid was transformed into *S. cerevisiae* Y2805 and Y2805Δgal80 strains. The ORF2 protein formed virus-like particle (VLP) in the cytoplasm of *S. cerevisiae* as observed by electron microscopy. The secretion of capsid protein into the culture supernatant was, however, unsuccessful. Purification of the VLP using various column chromatography was almost impossible due to the formation of large aggregates. Whole yeast containing ORF2 was immunized to guinea pigs and then serum titer was measured by ELISA. The antibody titer T/C values were higher than 2.0.

Conclusions

The ORF2 gene of porcine circovirus was expressed in yeast and it was observed that the VLP was efficiently formed. The feasibility of use of whole yeast cells containing the VLP as vaccine was tested in guinea pig experiments. In the ELISA test, the whole yeast cell containing VLP was found to be immunogenic. The recombinant ORF2 expressed in yeast is expected to be a candidate for a subunit vaccine for porcine circovirus.

FEMS7-0829
Pathogens / Pathogenicity - Part III

RECOMBINANT PRODUCTION OF SPAA, A SUBUNIT VACCINE FOR SWINE ERYSIPELAS, IN SACCHAROMYCES CEREVISIAE

S.H. Seo[1], K.M. Park[1], Y.J. Shim[2], S.S. Yoo[2], J. Ahn[1], E.S. Choi[1]
[1]Korea Research Institute of Bioscience & Biotechnology, Bio Process Engineering Center, Cheongju-si, Republic of Korea
[2]ChoongAng Vaccine Laboratories Co.- Ltd., Manufacture&Research, Daejeon, Republic of Korea

Backgrounds

A surface protein of *Erysipelothrix rhusiopathiae*, SpaA is a promising subunit vaccine against swine erysipelas. Recombinant production of SpaA was studied in yeast *Saccharomyces cerevisiae*. *S. cerevisiae* is a GRAS (generally regarded as safe) microorganism that has been exploited for thousands of years for fermentation, thus proven to be safe.

Objectives

To efficient produce SpaA for use as a subunit vaccine for swine erysipelas, using yeast expression systems including intracellular and surface-display expression.

Methods

For intracellular expression of SpaA, the SpaA gene that was chemically synthesized according to yeast codon preference, placed under GAL10 promoter and the resulting plasmid was transformed into *S. cerevisiae* Y2805 and Y2805Δgal80 strains. Since SpaA is a surface protein in *E. rhusiopathiae*, rSpaA was also expressed as surface-displayed protein. For surface display, SpaA gene was fused to the mating-factor alpha secretion signal sequence and an anchor-motif of a cell wall protein gene was fused at C-terminus of SpaA. The expression was confirmed by Western blot and the localization of the surface-displayed protein was monitored by fluorescence microscopy. The vaccine efficacy was evaluated by challenging mouse with virulent strain of *E. rhusiopathiae*.

Conclusions

The SpaA gene was expressed successfully both in cytoplasm and at the cell surface. The surface-display of the protein was confirmed by fluorescence microscopy. In mouse experiment, the crude preparation of SpaA from yeast lysate leads to 80% survival of mouse against lethal challenge with *E. rhusiopathiae*. The recombinant SpaA expressed in yeast is expected to be a useful candidate for a subunit vaccine for erysipelas.

FEMS7-2597
Pathogens / Pathogenicity - Part III

MOSAICISM OF HORIZONTALLY TRANSFERRED POTENTIAL MOBILE UNITS IN THE GENOME OF 'CANDIDATUS PHYTOPLASMA SOLANI' STRAIN SA-1

M. Seruga Music[1], I. Samarzija[1], S.A. Hogenhout[2], M. Haryono[3], C.H. Kuo[3]
[1]University of Zagreb, Department of Biology, Zagreb, Croatia
[2]John Innes Centre, Department of Cell and Developmental Biology, Norwich, United Kingdom
[3]Academia Sinica, Institute of Plant and Microbial Biology, Taipei, Taiwan R.O.C.

Backgrounds

Small and repeat-rich genomes are characteristic for the members of the genus '*Candidatus* Phytoplasma', endocellular phytopathogens transmitted and hosted by phloem-feeding insects. While phytoplasma economical importance is increasing, experimental studies are still lagging due to the inability to grow a pure phytoplasma culture. For most phytoplasma species, one of the hallmarks of their behaviour is the adaptability to different plant and insect hosts, especially in '*Ca*. P. solani' that has a broad host range as is transmitted by polyphagous insects.

Objectives

The aim of this work was to learn about the background of its plasticity and adaptability by sequencing the genome of '*Ca.* P. solani' strain SA-1.

Methods

Whole plant samples of infected periwinkle were used for generation of libraries sequenced on the Illumina MiSeq platform. *De novo* assembly generated the draft genome of 19 contigs with a total size of 821,322 bp. Repetitiveness and abundance of potential mobile units (PMUs) was confirmed and found responsible for the incomplete genome assembly. Moreover, a mosaicism of PMUs and PMU-like regions was found, corroborated both by the analyses of PMU organization and gene content as well as the molecular phylogeny of PMU-related genes *(dnaB, dnaG, hflB, tmk, fliA, tra5*).

Conclusions

This finding suggests a horizontal transfer of PMUs from related ('*Ca*. P. australiense') and more distant ('*Ca*. P. asteris' and '*Ca*. P. mali') phytoplasmas. We hypothesize that SA-1 strain gained a mosaic of PMUs, a trait not previously described for phytoplasma genomes, allowing high genome plasticity and successful adaptation to various plant and insect hosts.

FEMS7-0830
Pathogens / Pathogenicity - Part III

PATHOGENICITY FACTORS IN ACHROMOBACTER RUHLANDII ADAPTATION TO THE AIRWAYS OF CYSTIC FIBROSIS PATIENTS

N. Sharapova[1], M. Kunda[1], O. Voronina[1], A. Gintsgurg[1]
[1]N.F. Gamaleya Federal Research Centre for Epidemiology and Microbiology, Ministry of Health of the Russian Federation, Moscow, Russia

Backgrounds

Achromobacter ruhlandii SCCH3:Ach33-1365 (Aruh ST36) is Russian epidemic strain especially dangerous for cystic fibrosis (CF) patients because of transmissibility, multi-drug resistance and failure of eradication. Comparative analysis of virulence factors involved in adhesion, invasion and biofilms formation (pili, flagellar and bacterial capsule) could explain the extraordinary Arul ST36 pathogenicity.

Objectives

Aruh ST36 (Accession Number CP017433) was compared with two *Achromobacter* strains isolated from a CF patient: *A. xylosoxidans* MN001 (CP012046.1), USA, and *A. xylosoxidans* NH-44784-1996 (NC_021285.1), Denmark. According to MLST analysis MN001 was *A. ruhlandii* ST36 too. In each genome we revealed 18 pili formation genes assembled in the single *tad-rcp* operon; 56 flagellar genes organized in 8 operons and 10 bacterial capsule biosynthesis genes. 88% of all detected virulent factors were identical in the *A. ruhlandii* pair and only 9.5% - in the pair Aruh ST36 - NH-44784-1996. 13 proteins participated in *A. ruhlandii* ST36 adaptation to the local environmental conditions: ten flagellar proteins (4 chemotaxis, 3 regulatory proteins, M-ring protein, motor rotation protein MotA and secretion system III protein), one pili formation protein (peptidase TadV) and two proteins involved in bacterial capsule biosynthesis (polyketide synthase and WcbD).

Methods

454 Roche technology was used for Arul ST36 whole genome sequencing and assembling; RAST v.2.0 server – for genome annotation; VFDB (Virulence Factor DataBase) – for the virulence factors revealing. *Burkholderia cenocepacia* strain J2315 (NC_011000.1) has the closest taxonomic relationship with *Achromobacter* in VFDB.

Conclusions

Flagellar proteins are the most important virulence factors in adaptation of *A. ruhlandii* epidemic strain.

FEMS7-2240
Pathogens / Pathogenicity - Part III

PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG GENERAL POPULATION IN NORTH OF IRAN

M. Sharif[1], A. Ahmad Daryani[1], M. Mohtaram Nasrolahei[2], S. Shahabeddin Sarvi[1], A.S. Abdol Sattar Pagheh[1]
[1]Toxoplasmosis Research Center- Mazandaran University of Medical Sciences- Sari- Iran, Parasitology and Mycology, Sari, Iran
[2]Microbiology Department- Faculty of Medicine- Mazandaran University of Medical Sciences- Sari- Ira n, microbiology, Sari, Iran

Backgrounds

Intestinal parasitic infection (IPIs) is one of the most important health problems in developing countries. The prevalence of these infections is different among various communities; hence, there is a need for the periodical prevalence evaluation to develop public health strategies.

Objectives

The study aimed to investigate the prevalence and risk factors of intestinal parasites among the general population of Mazandaran province in north of Iran, as the first comprehensive research in this region.

Methods

This cross-sectional study was conducted in general population in Mazandaran province, north of Iran from December 2015 to December 2016. Faecal samples were selected from 17 urban areas (2515 samples) and 34 rural areas (2273 samples). 4788 specimens from 2579 males and 2579 females were examined by direct wet mounting, formalin-ether concentration, and Ziehl–Neelsen and trichrome permanent staining methods.

Conclusions

Intestinal parasites were found in 680 (14.2%) of the studied samples. Blastocystis hominis (5.2%) and Giardia lamblia (4.6%) were the most frequent parasites. Protozoa, helminthes and mix infections were observed in 12.32%, 1.03% and 0.85 of specimens, respectively. A significant association was observed with household income, place of residence, kind of washing vegetables, contact with soil and season. But neither age and nor gender were correlated to parasite infection.These findings emphasized that IPIs are a health concern in this area, so programs including screening and treatment of patients, improving sanitary conditions and public education for preventing the spread of intestinal parasitic are required.

FEMS7-0195
Pathogens / Pathogenicity - Part III

ALCOHOL AND TOBACCO CONSUMPTION AFFECT THE ORAL CARRIAGE OF CANDIDA ALBICANS AND MUTANS STREPTOCOCCI

C. Sheth[1], M.D.M. Jovani-Sancho[2], R. Gonzalez[2], V. Veses[3]
[1]Universidad CEU Cardenal Herrera, Medicine, Alfara de Patriarca, Spain
[2]Universidad CEU Cardenal Herrera, Dentistry, Alfara de Patriarca, Spain
[3]Universidad CEU Cardenal Herrera, Biomedical Sciences, Moncada, Spain

Backgrounds

The consumption of alcohol and tobacco is widespread in modern society. This study defines the relationship between the consumption of these social stimulants and the oral colonization by mutans streptococci and Candida species. Both species are common commensals in the oral cavity and both are implicated in disease processes (Candida; oropharyngeal candidiasis, denture-related stomatitis and Streptococcus; caries, infective endocarditis).

Objectives

The study had two main objectives:
1. Investigate changes in oral flora in males vs females induced by the consumption of alcohol and tobacco
2. Compare the differences in oral flora composition as a result of long versus short term consumption of alcohol and tobacco

Methods

Subjects were recruited from the University Dental Clinic of CEU Cardenal Herrera University (Moncada, Valencia). Information about alcohol and tobacco consumption was obtained by questionnaire. Individual stimulated saliva samples were obtained and selective media was used to isolate and quantify the colony forming units per mililiter of saliva (CFU / ml) of mutans streptococci and Candida spp.

Conclusions

Alcohol consumption statistically significantly decreased oral carriage of mutans streptococci ($p= 0.005$). Tobacco users were found to harbour elevated levels of C. albicans ($p= 0.03$). We observed changes in the levels of microbial colonization in response to the consumption of alcohol and tobacco in a species-specific manner. Whereas smoking increases the levels of Candida albicans in the oral cavity, drinking alcohol reduces the levels of mutans streptococci. Other species investigated, such as Candida krusei, Candida tropicalis and Lactobacilli, do not show a response to the consumption of the stimulants analyzed.

FEMS7-1471
Pathogens / Pathogenicity - Part III

IL-6 AMELIORATES ACUTE LUNG INJURY IN INFLUENZA VIRUS INFECTION

A.L. Shiau[1], Y. Mei-Lin[1], W. Chao-Liang[2]
[1]National Chen Kung University Medical College, Department of Microbiology and Immunology, Tainan, Taiwan R.O.C.
[2]National Chen Kung University Medical College, Department of Biochemistry and Molecular Biology, Tainan, Taiwan R.O.C.

Backgrounds

Interleukin 6 (IL-6) is involved in innate and adaptive immune responses to defend against pathogens. Although the role of IL-6 in influenza pathogenesis has been documented, to date no studies have investigated its role in modulating lung repair responses necessary for recovery from influenza.

Objectives

We studied the role of IL-6 in lung repair from influenza-induced lung injury in mice.

Methods

We used IL-6 knockout and wild-type C57BL/6 mice to establish an influenza-induced acute lung injury model. We investigated the effects of IL-6 on the functions of lung fibroblasts, epithelial cells, and macrophages, as well as the interplay among these cell types during influenza infection. Immunohistochemical, immunological, apoptotic, migration, and phagocytosis assays were employed.

Conclusions

IL-6-deficient mice infected with influenza virus exhibited higher lethality, lost more body weight, and had higher fibroblast accumulation, and lower extracellular matrix (ECM) turnover in the lung than their wild-type counterparts. Deficiency in IL-6 enhanced proliferation, migration, and survival of lung fibroblasts, as well as increased virus-induced apoptosis of lung epithelial cells. IL-6-deficient lung fibroblasts produced elevated levels of TGF-β, which may contribute to their survival. Macrophage recruitment to the lung and phagocytic activities of macrophages during influenza infection were reduced in IL-6-deficient mice.

Our results indicate that IL-6 is crucial for lung repair after influenza-induced lung injury through reducing fibroblast accumulation, promoting epithelial cell survival, increasing macrophage recruitment to the lung, and enhancing phagocytosis of viruses by macrophages. This study suggests that IL-6 may be exploited for lung repair during influenza infection.

FEMS7-1361
Pathogens / Pathogenicity - Part III

EPIDEMIOLOGICAL STUDY OF MULTIDRUG-RESISTANT PSEUDOMONAS AERUGINOSA ISOLATES IN HAMAD MEDICAL CORPORATION, QATAR

M. Sid Ahmed[1], J. Jass[1], E. Ibrahim[2], B. Söderquist[3]
[1]Örebro University, School of Science and Technology, Örebro, Sweden
[2]Hamad Medical Corporation, The Department Laboratory Medicine and Pathology- Microbiology, Doha, Qatar
[3]Örebro University, School of Medical Sciences, Örebro, Sweden

Backgrounds

Nosocomial infections affect millions of patients yearly. Gram-negative bacteria (GNB) deserve special attention as some have inherited resistance to all available antibiotics, leaving only the more toxic agents (e.g. polymyxins) for treatment. *Pseudomonas aeruginosa* is the second most common GNB isolated from health care infection. There is insufficient data about the epidemiology of multidrug- and pandrug-resistant *P. aeruginosa* (MDR-PA) in Qatar.

Objectives

The aim of the present study was to determine antimicrobial susceptibility patterns and epidemiology of MDR-PA from patients from Hamad Medical Corporation, Qatar

Methods

A total of 2552 *P. aeruginosa* isolates were identified from 5 hospitals in Qatar and antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute. Clinical data were collected prospectively, including electronic medical records

Conclusions

The overall prevalence of MDR-PA isolates was 8% (n=205). Majority of the patients were male (75%), aged >50 years (64%) and recently hospitalized (74%). Among the infected patients with MDR-PA, 85% of were exposed to antibiotics during the last 90 days and 97% were hospital acquired while 3% were community acquired. However, of the MDR-PA, 56% were due to colonization, while 44% were infections. Most of the MDR-PA (96.6%) isolates were resistant to cefepime, 91% to ciprofloxacin, 91% to piperacillin/tazobactam and 90% to meropenem. Majority of MDR-PA isolates also exibited universal sensitivity to colistin, the drug of last resort treatment, thus considered pan-resistant. Our study showed a higher incidence of MDR-PA in comparison to neighboring countries, providing insight into the current situation in Qatar.

FEMS7-2652
Pathogens / Pathogenicity - Part III

STUDYING THE ROLE OF CGMIP1 IN THE EVOLUTION OF CANDIDA GLABRATA DURING ADAPTION TO THE HUMAN HOST

S. Siscar Lewin[1], *S. Brunke*[1], *B. Hube*[1,2,3]
[1]*Department of Microbial Pathogenicity Mechanisms,*
Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), Jena, Germany
[2]*Center for Sepsis Control and Care, Jena University Hospital, Jena, Germany*
[3]*Friedrich Schiller University, Jena, Germany*

Backgrounds

Candida glabrata is the second most prevalent cause of candidemia worldwide, which is largely due to its high intrinsic and rapidly acquired resistance to azole antifungals. Phylogenetically, *C. glabrata* is more closely related to *Saccharomyces cerevisiae* than to the most common *Candida* species, *C. albicans*. Recent evolutionary studies have shown that the gene *CgMIP1* may have been under positive selection during the evolution of *C. glabrata* as human pathogen. The ortholog *MIP1* in *Saccharomyces cerevisiae* encodes a mitochondrial polymerase. Defects in mitochondrial functions lead to respiratory deficiency, as described in *petite* mutants, and increased resistance against azole antifungals.

Objectives

We studied the possible role of *CgMIP1* in *C. glabrata* pathogenicity compared to the non-pathogenic species *S. cerevisiae.*

Methods

We created *MIP1* knock-out mutants of both species and analyzed their growth under different stress conditions, such as oxidative stress, osmotic stress, endoplasmic reticulum (ER) stress and cell wall stress.

Conclusions

MIP mutants (*mip1Δ*) of both species showed *petite*-like phenotype. Interestingly, the *C. glabrata* mutant showed steady growth under ER stress conditions, whereas the growth of the wild type and *S. cerevisiae* strains was notably slower. Hence, deletion of *MIP1* conferred a species-specific increased resistance to these stressors for *C. glabrata*.

These adaptations might originally be due to frequent exposure to noxious substances, e.g. originating from the mucosal flora, which made selection pressure on *CgMIP1*. This could allow loss of function upon certain stressors and better survival in *petite*-like growth. Further studies are underway to verify this hypothesis.

FEMS7-2283
Pathogens / Pathogenicity - Part III

MOLECULAR CHARACTERIZATION OF VANCOMYCIN-RESISTANT ENTEROCOCCI AT UNIVERSITY HOSPITALS IN BRNO (CZECH REPUBLIC)

M. Sittová[1], M. Röderová[2], M. Dendis[1], O. Holý[3], F. Růžička[4]
[1]GeneProof a.s., Research and Development, Brno, Czech Republic
[2]Faculty of Medicine and Dentistry- Palacký University Olomouc, Microbiology, Olomouc, Czech Republic
[3]Faculty of Medicine and Dentistry- Palacký University Olomouc, Preventive Medicine, Olomouc, Czech Republic
[4]Faculty of Medicine- St. Anne's University Hospital- Masaryk University, Microbiology, Brno, Czech Republic

Backgrounds

Enterococci are Gram-positive bacteria occurring as part of the natural microflora in the gastrointestinal tract. Vancomycin-resistant enterococci (VRE) represent a growing threat in hospital-acquired infections.

Objectives

The aim of this study was to monitor the incidence of VRE at the particular university hospitals in Brno (Czech Republic) in the period January-September 2015 where the increasing trend of VRE has occurred. Determination of virulence factors, detection of genes for antimicrobial resistance and assessment of their clonality were also part of this study.

Methods

Enterococci were isolated from various kinds of clinical specimens. They were identified using standard microbiological methods and MALDI-TOF MS. Determination of antibiotic susceptibility was performed by disk diffusion test. Real-time PCR was used for *vanA* gene and *vanB* detection. Genes for virulence factors were examined by end-point PCR: gelatinase (*gel*), surface protein (*asp*) cytolyzin (*cylA*), aggregation substance (*asa1*) and hyaluronidase (*hyl*). The clonality of individual strains was determined by pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested DNA.

Conclusions

In the reporting period 32 VRE isolates from 26 patients were collected. All isolates were identified as *Enterococcus faecium* with *vanA* gene. The most common clinical material was urine (17). Only *esp* and *hyl* were detected from virulence factors. *Esp* gene was detected in eight samples (25%), *hyl* gene in 13 samples (41 %) and both genes (*esp*, *hyl*) in nine samples (28%). None of virulence factors was detected at two samples (6%). All isolates were resistant to vancomycin and aminopenicillin. Sensitivity to linezolid and tigecycline remained in all specimens. PFGE revealed identical strains with possible clonal spreading only in four patients (5 strains). Although, there is an increasing trend in incidence of VRE, PFGE results did not reveal any significant distribution of identical strains within selected hospitals in Brno. We assume that it is due to correct hospital infection control.

FEMS7-1324
Pathogens / Pathogenicity - Part III

THE IMPACT OF PUTATIVE VIRULENCE GENES ON PATHOGENICITY DISPLAYED BY CLINICAL ACINETOBACTER BAUMANNII STRAINS

J. Skerniskyte[1], E. Karazijaite[1], J. Deschamps[2], R. Briandet[2], C. Pechoux[3], S. Kulakauskas[2], R. Krasauskas[1], J. Armalyte[1], E. Suziedeliene[1]
[1]Life Sciences Center- Vilnius University, Institute of Biosciences, Vilnius, Lithuania
[2]INRA- AgroParisTech- Universite Paris-Saclay, Micalis Institute, Jouy-en-Josas, France
[3]INRA- AgroParisTech- Universite Paris-Saclay, GABI, Jouy-en-Josas, France

Backgrounds

Acinetobacter baumannii is one of the major infection agents causing nosocomial pneumonia. Bacterium displays a high degree of genome plasticity which is thought to enable its successful adaptation to clinical environment and within the host. The impact of genome features on the virulence-associated properties of clinical *A. baumannii* strains is little investigated.

Objectives

To access virulence properties displayed by clinical *A. baumannii* strains and to investigate the role of putative virulence genes in bacterium pathogenicity with an emphasis on the bacterial surface-related properties.

Methods

Clinical *A. baumannii* strains (n=140) were screened for the genome presence of candidate virulence genes. Representative strains were further tested for biofilm formation, hydrophobicity, serum resistance, adhesion to mucin and to lung epithelial cells, virulence in *Caenorhabditis elegans* model. *A. baumannii* deletion mutants of putative virulence genes were generated by natural recombination. Biofilm structure was analyzed by confocal laser microscopy, cell wall changes were accessed by transmission electron microscopy.

Conclusions

Of the 10 *A. baumannii* candidate genes tested, the distribution of genes coding for bacterial surface-related proteins *blp1*, *smpA*, *epsA*, *bap*, *acfC* showed specific association with the genomes of pandemic clonal lineages I or II suggesting that alternative mechanisms might contribute to the environmental fitness of most successful *A. baumannii* clones. Moreover, the significant variation in virulence phenotypes was observed among individual *A. baumannii* strains. *blp1* and *ompA* gene mutants showed the most attenuated expression of virulence-associated features including changes in biofilm and cell wall structures.

FEMS7-1685
Pathogens / Pathogenicity - Part III

CONTRIBUTION OF CARBAPENEMASES AND EFFLUX PUMPS TO CARBAPENEM RESISTANCE IN ACINETOBACTER BAUMANNII CLINICAL ISOLATES FROM POLAND

A. Słoczyńska[1], *A.E. Laudy*[1], *S. Tyski*[1,2]
[1]*Medical University of Warsaw, Department of Pharmaceutical Microbiology, Warsaw, Poland*
[2]*National Medicines Institute, Department of Antibiotics and Microbiology, Warsaw, Poland*

Backgrounds

Acinetobacter baumannii is recognized as important cause of nosocomial infections worldwide.

Objectives

The aim of the study was to determine carbapenem resistance mechanisms of Polish *A.baumannii* clinical isolates.

Methods

A total of 62 *A.baumannii* clinical isolates from Polish hospitals were analyzed for mechanisms of carbapenem resistance. Species identification was confirmed by *gyrB* PCR. All isolates were defined as imipenem resistant/non-susceptible by disk diffusion method. PCR was used to detect carbapenem hydrolyzing class D carbapenemases (CHDL) genes (*bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{OXA-58-like}}$) with insertion sequences (IS). All isolates were subjected to CarbAcineto test. MICs for imipenem and meropenem, also in the presence of efflux pump inhibitor PAβN (50 µg/ml), were determined using broth dilution method.

Conclusions

All 62 isolates possessed the intrinsic *bla*$_{\text{OXA-51}}$ gene, while 57 (91,9%) harbored simultaneously also one of the acquired CHDL genes. 40/62 isolates (64,5%) harbored *bla*$_{\text{OXA-24}}$, 14/62 (22,6%) *bla*$_{\text{OXA-23}}$ and 3/62 (4,8%) *bla*$_{\text{OXA-58}}$. IS were detected upstream of all *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-58}}$ genes. Highest meropenem and imipenem MICs (up to 128 µg/ml and 64 µg/ml, respectively) were observed for OXA-24-positive isolates. In the case of OXA-58 and OXA-51-positive isolates the lowest carbapenem MIC (4 µg/ml) was observed, which was concordant with negative CarbAcineto test results for these isolates. PAβN reduced by at least 4-fold meropenem MIC of 10 (71,4%) OXA-23 positive isolates, 4 (10%) OXA-24 and 1 (33,3%) OXA-58 positive isolate. Obtained results suggest that carbapenem resistance of Polish *A.baumannii* isolates is mainly mediated by acquired carbapenemases, however efflux pump activity may also play a role in this resistance profile.

FEMS7-0673
Pathogens / Pathogenicity - Part III

MULTISTATE SEPSIS OUTBREAK CAUSED BY ENTEROBACTERIACEAE IN BRAZIL LED TO IDENTIFICATION OF CLINICALLY RELEVANT PHYTOBACTER SPP. AS CAUSE FOR PAST AND PRESENT EPIDEMICS

M. Pillonetto[1], L. Arend[2], H. Faoro[3], H. D'Espindula[1], J. Blom[4], T. Smits[5], M. Mira[1], F. Rezzonico[5]
[1]Core for Advanced Molecular Investigation- Graduate Program in Health Sciences, School of Medicine- Pontifícia Universidade Católica do Paraná, Curitiba, Brazil
[2]Molecular Bacteriology Division, Central Public Health Laboratory – State of Paraná, São José dos Pinhais, Brazil
[3]Carlos Chagas Institute, FIOCRUZ, Curitiba, Brazil
[4]Bioinformatics and Systems Biology, Justus-Liebig-University, Giessen, Germany
[5]Research Group Environmental Genomics and Systems Biology- IUNR, Life Sciences and Facility Management, Wädenswil, Switzerland

Backgrounds

Twenty-five clonal Gram-negative, rod-shaped, non-spore-forming bacteria were isolated from total parenteral nutrition during a 2013 multistate sepsis outbreak in Brazil. Standard clinical identification protocols pointed to the former *Erwinia herbicola-Enterobacter agglomerans* complex (EEC), but yielded contradictory results.

Objectives

Taxonomic description of the outbreak organism.

Methods

Multi-locus sequence analysis (MLSA) of representative isolate 5110RM suggested that the latter was closely related to *Phytobacter diazotrophycus* DSM 17806^T, the type strain of a non-validly described species isolated from wild rice in China, and to a number of clinical strains isolated in the United States in the 1970's that, based on DNA similarity, were previously assigned to Biotype XII of the EEC.

Conclusions

Both MLSA and whole-genome sequence data analysis supported the existence of two separate species within the genus *Phytobacter* as well as the distinctiveness of the latter from nearby genera such as *Kosakonia* or *Citrobacter*. The name *Phytobacter ursingii* sp. nov. was proposed for the second species. While both species contain plasmid-based multidrug resistant isolates, their relevance for clinical surveillance is given, moreover that isolates from this genus were repeatedly misidentified as *Pantoea agglomerans* in the past. We are currently designing assays to detect the genus *Phytobacter* from clinical samples. This will enable to study its diversity, spread and will help to prevent further septicaemia outbreaks by their implementation in surveillance programs.

FEMS7-2834
Pathogens / Pathogenicity - Part III

ANTIBIOTIC SENSITIVITY OF BACTERIA CONTAMINATING BOAR SEMEN EXTENDED SAMPLES AND EFFICACY OF COMMERCIAL COLLECTION EXTENDER

P. Soler-Llorens[1], R. Ausejo[1], N. Mendoza[1], J. Miguel[1]
[1]Magapor S.L., R&D - Veterinary service, Ejea de los Caballeros, Spain

Backgrounds

Artificial Insemination (AI) is the predominant form for breeding in swine. Semen is diluted in extender in order to preserve its viability for a period of time. Bacterial contamination of extended porcine semen occurs frequently in AI centers, and may occur during whole semen processing.

Objectives

The aims of this study were to check the main bacterial species isolated from seminal doses; to assess the activity of antibiotics and the appropriate combination to control bacteria; and to evaluate the efficacy of a collection extender, Dicol®, in controlling contamination.

Methods

Samples were collected from different Spanish AI centers. Bacterial culture, isolation, identification and antibiogram of the isolated bacteria were performed. Antibiotic-free extender containing semen samples were aliquoted and infected with 10^6 ufc/ml of *Serratia marcescens* (Gram-negative), and *Micrococcus spp*. (Gram-positive). Infected semen samples were and incubated with 4 concentrations of 10 antibiotics and cultured for bacterial content. Ejaculates from five different boars were collected and divided into 3 groups which were initially diluted (1:1) with Dicol, Vitasem or Duragen extender, aliquoted and infected with 10 multi-resistant bacterial isolates. After 25 or 50 min, final dilution was carried out and stored at 16ºC and bacterial load determined at 24 hours post-infection.

Conclusions

Most frequent bacteria present on samples were: *Proteus vulgaris* (3.5%), *Serratia marcescens* (6.19%), *Serratia liquefaciens* (12.39%), *Stenotrophomonas maltophilia* (7,96%), *Staphylococcus spp.* (9.4%), Cedea (4.4%), *Micrococcus spp*. (5.3%), *Morganella mornanii* (4.4%). Dicol® treated samples showed almost no growth (<1cfu/ml), even when the final dilution was in with antibiotics-free extender.

FEMS7-2841
Pathogens / Pathogenicity - Part III

CHARACTERIZATION OF AN ATYPICAL BRUCELLA SPP. ISOLATE FROM A PAC-MAN FROG (CERATOPHRYS ORNATA) SHOW CHARACTERISTICS DEPARTING FROM CLASSICAL BRUCELLAE

P. Soler-Lloréns[1,2], C.R. Quance[3], S.D. Lawhon[4], T.P. Stuber[3], J.F. Edwards[4], T.A. Ficht[5], S. Robbe-Austerman[3], D. O'Callaghan[1,2], A. Keriel[1,2]
[1]Institut National de la Santé et de la Recherche Médicale, U1047- UFR de Médecine, Nîmes, France
[2]Université de Montpellier, U1047, Nîmes, France
[3]Animal and Plant Health Inspection Service- United States Department of Agriculture-, Mycobacteria and Brucella Section- National Veterinary Services Laboratories, Ames- IA, USA
[4]College of Veterinary Medicine and Biomedical Science- Texas A&M University, Clinical Microbiology- Department of Veterinary Pathobiology, College Station- TX, USA
[5]Texas A&M University, Department of Veterinary Pathobiology, College Station- TX, USA

Backgrounds

Brucella are highly infectious bacterial pathogens responsible for brucellosis, a frequent worldwide zoonosis. The *Brucella* genus has recently expanded from 6 to 11 species, all of which were associated with mammals; The natural host range recently expanded to amphibians after some reports of atypical strains from frogs.

Objectives

Here we describe the first in depth phenotypic and genetic characterization of a *Brucella* strain isolated from a frog. Strain B13-0095 was isolated from a Pac-Man frog (*Ceratophyrus ornate*) at a veterinary hospital in Texas and was initially misidentified as *Ochrobactrum anthropi*.

Methods

Whole genome sequencing was performed for B13-0095. The result was assembled and annotated. *In Silico* analysis of the sequence revealed several specific features, which were evaluated by several methods (motility assays, growth under different conditions, SDS-PAGE, or *in vitro* infections).

Conclusions

B13-0095 belongs to a group of early-diverging brucellae that includes *Brucella inopinata* strain BO1 and the *B. inopinata*-like strain BO2. B13-0095 genome sequence revealed specific features that suggest that this isolate represents an intermediate between a soil associated ancestor and the host adapted "classical" species. Like strain BO2, B13-0095 does not possess the genes required to produce the perosamine based LPS found in classical Brucella, but has a set of genes that could encode a rhamnose based O-antigen. B13-0095 has a very fast intracellular replication rate in epithelial cells and macrophages. Finally, the study also shows that B13-0095, BO1, and BO2 are motile strains, which is remarkable for this bacterial genus.

FEMS7-0138
Pathogens / Pathogenicity - Part III

ECTOPIC EXPRESSION OF PAR-4 LEADS TO INDUCTION OF APOPTOSIS IN MYCOBACTERIA-INFECTED MACROPHAGES

Y.J. Lim[1], J.A. Choi[1], J.H. Lee[1], S.N. Cho[1], S.M. Oh[1], D. Go[1], S.H. Kim[1], C.H. Song[1]
[1]Chungnam National University - School of Medicine,
Department of Microbiology- Department of Medical Science, Daejeon, Republic of Korea

Backgrounds

Immunotherapy with mycobacteria has been used successfully as a treatment for bladder and prostate cancers. Bacillus Calmette-Guérin (BCG) is a strain of *Mycobacteria bovis* that effectively induces an immune response against tumor cells, leading to a reduction of risk factors associated with tumor recurrence and progression. However, the exact mechanisms of mycobacterial immunotherapy are not fully understood. Prostate apoptosis response-4 (Par-4) is a protein that was first identified in prostate cancer cells undergoing apoptosis. Although Par-4 is incapable of directly inducing apoptosis in normal cells, Par-4 overexpression is sufficient to induce apoptosis in most cancer cells. Secretion of Par-4 follows the conventional ER-Golgi secretory pathway, and is associated with ER stress.We hypothesize that *Mtb*-induced GRP78 production coordinates with Par-4 to induce apoptosis of *Mtb*-infected macrophages.

Objectives

The aim of this study was to investigate the role of ectopic expression of Par-4 during mycobacterial infection.

Methods

In this study, we measured Par-4 levels by Western blot analysis of RAW 264.7 macrophages infected with the H37Ra strain of *Mtb*. We examined Par-4 production in *Mtb* H37Ra-infected macrophages treated with the chemical chaperone and inhibitor of ER stress. To investigate the role of ectopic exressed Par-4, we visualized Par-4 localization in *Mtb*-infected macrophages and analyzed apoptotic cell death.

Conclusions

Par-4 is associated with ER stress-induced apoptosis resulting in reduced intracellular survival of mycobacteria. BCG treatment increases Par-4-dependent caspase activation in prostate cancer cells. Therefore, ER stress-induced Par-4 acts as an important defense mechanism against mycobacterial infection and regulates cancer.

FEMS7-0978
Pathogens / Pathogenicity - Part III

BACTERIAL PATHOGENS DETECTED IN CYSTIC FIBROSIS PATIENTS

V. Spendal[1], *N. Fajfar*[1], *D. Luznik*[1], *V. Tomic*[1]
[1]*University Clinic of Respiratory and Allergic Diseases Golnik, Laboratory for respiratory microbiology, Golnik, Slovenia*

Backgrounds

Cystic fibrosis (CF) is the most common genetic disease. In CF a defect (mutation) in a gene changes a protein that regulates the movement of salt in and out of cells. The result is thick, sticky mucus in the lungs which provides an ideal breeding ground for bacteria.

Objectives

The objective of this study was to characterize the bacterial pathogens colonizing lungs of patients with CF during ten-year period.

Methods

In the Laboratory for Respiratory Microbiology 471 clinical isolates were collected from 31 patients with CF, who were admitted to University Clinic of Respiratory and Allergic Diseases Golnik between 2006 and 2016. In 31 patients (21 female, 10 male) we found 13 different potentially pathogenic bacteria. The most commonly isolated pathogen was *Staphylococcus aureus* found in 77,4% of patients (90 % male, 77 % female), followed by *Pseudomonas aeruginosa* 54,8 % of patients (40% male, 62 % female). *Haemophilus influenzae* and *Bulkholderia cepacia complex* were isolated in 16,1 % of patients. *Stenotrophomonas maltophilia* was isolated from 9,7 % patients. *Moraxella catarrhalis*, *S. pneumoniae, Streptococcus group C, Klebsiella pneumoniae* and *Escherichia coli* were found in 3,2 % patients. In two cases we isolated methicillin-resistant *S. aureus* (MRSA).

Conclusions

The results showed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most commonly isolated organisms from the respiratory tract of these CF patients. Multidrug resistant organisms were uncommon.

FEMS7-1865
Pathogens / Pathogenicity - Part III

THE EXTRACELLULAR ACID TREHALASE ENCODED BY THE ATH1 GENE IS REQUIRED FOR VIRULENCE OF CANDIDA GLABRATA

R.G. Lopes[1], J.E. Muñoz[2], L.M. Barros[1], C.P. Taborda[2], B.U. Stambuk[1]
[1]Universidade Federal de Santa Catarina- Centro de Ciências Biologicas, Bioquímica, Florianopolis, Brazil
[2]University of São Paulo- Institute of Biomedical Sciences, Microbiology, São Paulo, Brazil

Backgrounds

Both the incidence of invasive fungal infections and rates of multidrug resistance associated with the fungal pathogen *Candida glabrata* have increased in recent years, accounting for as many as 10-25% of all candidaemias. The carbon utilization spectrum of this yeast is extremely narrow when compared with other yeasts (only consumes glucose and the disaccharide trehalose), and has been suggested to be consequence of an adaptation for its life and success as a commensal pathogen. Indeed, rapid identification tests for this species are based on the ability of this yeast to rapidly hydrolyze trehalose, but not other disaccharides.

Objectives

We have recently characterized the extracellular trehalase encoded by the *CgATH1* gene of *C. glabrata* (Microbiol. Res. 179: 12-19, 2015), which allows efficient hydrolysis and fermentation of this sugar by this yeast. One intriguing point is why this yeast has retained the ability to utilize extracellular trehalose as carbon source, since this sugar is not found easily in the environment or mammalian host.

Methods

Thus, we decided to delete this gene from the genome of *C. glabrata* using the promoter-dependent disruption of genes (PRODIGE) method.

Conclusions

Our results show that the *C. glabrata CgATH1*Δ::*ScURA3* mutant strain is, as expected, unable to consume and ferment extracellular trehalose. However, our results also show that deletion of this gene also led to a significant ($p<0.01$) reduction in virulence in a murine model of infection, using normal or immunocompromised (treated with cyclophosphamide) mice, indicating that trehalose metabolization is important for virulence in the mammalian host of this fungal pathogen.

FEMS7-0495
Pathogens / Pathogenicity - Part III

GENOME ANALYSIS OF NINE TREPONEMA PALLIDUM SUBSP. PERTENUE STRAINS

M. Strouhal[1], L. Mikalová[1], J. Haviernik[1], D. Čejková[2], P. Tenti[1], D. Šmajs[1]
[1]Masaryk University- Faculty of Medicine, Department of Biology, Brno, Czech Republic
[2]Veterinary Research Institute, Department of Immunology, Brno, Czech Republic

Backgrounds

Treponema pallidum subsp. *pertenue* (TPE) is the causative agent of yaws, a multistage disease endemic in tropical regions in Africa, Asia, Oceania, and South America. To date, only four TPE strains were completely sequenced and analyzed including three TPE strains of human origin (Samoa D, CDC-2, and Gauthier) and one TPE strain isolated from the baboon (Fribourg-Blanc).

Objectives

The aim of this study was genome analysis of five TPE strains of African and Indonesian origin (CDC-1, CDC2575, Ghana-051, Kampung Dalan, and Sei Geringging) and comparison of these complete genome sequences with other available TPE genomes.

Methods

The genomes were determined using the pooled segment genome sequencing method combined with Illumina sequencing platform. Whole genome nucleotide alignment of nine TPE strains was used for determination of genetic relatedness using several approaches including single nucleotide variants (SNV) determination, calculation of nucleotide diversity and construction of a phylogenetic tree.

Conclusions

There were a total of 443 identified SNV among TPE genomes (excluding *tprK* and *tprD* genes and 16S-23S rDNA intergenic regions). Most of them represented strain-specific SNV. No larger genome rearrangements were found. The nucleotide sequences of strains CDC2575 and Ghana-051 were found to be identical differing only in intra-strain heterogenic sites. The nucleotide diversity (π) ranged between 0,00006±0,00003 – 00040±0,00020. Phylogenetic tree showed no identifiable subgroups among analyzed TPE strains suggesting unique ancestor of TPE strains.

FEMS7-1895
Pathogens / Pathogenicity - Part III

TOPOLOGICAL MODEL FOR THE SEARCH OF NEW ANTIBACTERIAL COMPOUNDS. FIRST ASSAYS

B. Suay[1], J.I. Bueso-Bordils[1], M.T. Perez-Gracia[1], G. Anton-Fos[1], L. Lahuerta-Zamora[1], R. Martin-Algarra[1], P. Aleman-Lopez[1]
[1]University CEU Cardenal-Herrera, Pharmacy, Valencia, Spain

Backgrounds

Due to the increasing detection of multi-drug resistant bacteria, the arsenal of antibacterial compounds has to be constantly evolving. We developed a topological model to predict antibacterial activity.

Objectives

To verify experimentally our topological model by assaying a group of theoretically active compounds, randomly selected from said model. Moreover, the results obtained will allow verification and further optimization of this model as well as the identification of new lead compound.

Methods

Of the 158 theoretically active compounds, 14 were randomly selected to be assayed against four bacteria (*Staphylococcus aureus*, *Streptococcus agalactiae,* E*scherichia coli* and *Serratia marcescens*). All 14 compounds were tested for antibacterial activity using the Kirby-Bauer method, having impregnated the disks with a saturated solution of each compound. After obtaining these results, the compounds showing an inhibition zone were assayed following the CLSI standards to determine their Minimum Inhibitory Concentration (MIC).

Conclusions

Of the 14 compounds assayed, 4 presented bactericidal activity at saturated concentrations. Of these, only 1 showed a MIC below 512µg/mL, therefore becoming possible lead compound candidates. Furthermore, the negative results obtained provide information necessary to improve our topological model in order to obtain better results in the future. Therefore, we consider molecular topology to be a cost-effective, powerful and useful tool for the identification of new antibacterial compounds, which could be of interest against multi-drug resistant bacteria.

FEMS7-1467
Pathogens / Pathogenicity - Part III

BACTERIOPHAGES IN AGRICULTURE: OVERVIEW OF A PROGRAM TO DEVELOP A HIGH EFFICACY BIOPESTICIDE FOR CONTROL OF ERWINIA AMYLOVORA

A. Svircev[1], *A. Castle*[2]
[1]*Agriculture and Agri-Food Canada, STB, Vineland Station, Canada*
[2]*Brock University, Department of Biological Sciences, St. Catharines, Canada*

Backgrounds

Commercial biologicals are available for the control of *Erwinia amylovora*, the fire blight pathogen, in apple and pear orchards. In the blossom, the biologicals suppress the pathogen on the pistil by competing for nutrients and/or through the production of antimicrobial compounds. In contrast, lytic bacteriophages have the ability to infect the pathogen and destroy the infected host cells through cell lysis. In agricultural applications, bacteriophages are restricted by the adverse environmental conditions such as UV light and dry conditions.

Objectives

We have developed a biological that incorporates lytic phages inside the epiphytic bacterium, *Pantoea agglomerans*. The *P. agglomerans* performs as biological control agent and a bacteriophage the carrier.

Methods

Once applied to the blossom under optimal conditions results in an increase in both the phage and the carrier populations. In this phage-carrier mediated biological control system, both the bacteriophage host range and the development of bacterial resistance need to be considered. Phages were collected and screened for efficacy against the pathogen using detached flower bioassays, screen house and field trials. Ten phages demonstrated high field based efficacy.

Conclusions

This presentation will focus on the impact of bacterial resistance mechanisms on phage efficacy, specifically, the role of bacterial exopolysaccharides (amylovoran and levan) in phage infection and growth, the possible roles of lysogeny, and CRISPR/Cas systems on phage-host interaction and resistance.

FEMS7-1808
Pathogens / Pathogenicity - Part III

THE EFFECT OF PROTEOLYTIC ENZYMES INHIBITORS ON PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS CELLS

K. Szałapata[1], *M. Osińska-Jaroszuk*[1], *A. Jarosz-Wilkołazka*[1]
[1]*Maria Curie Sklodowska University, Department of Biochemistry, Lublin, Poland*

Backgrounds

Pseudomonas aeruginosa and *Staphylococcus aureus* are one of the main pathogens which cause postoperative prosthesis infections in many patients. Late detection or improperly treating of these infections leads to the need of a repeated surgery and sometimes even ends up the death of the patients. Currently we are looking for new substances which are more effective in action than the antibiotics. The efficient competition may be a group of natural and synthetic inhibitors of proteolytic enzymes, which are very important as homeostasis regulators.

Objectives

The aim of this study was to investigate the selected proteolytic enzyme inhibitors (AEBSF and α-1-antitrypsin) affecting the viability of two pathogenic strains - *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Methods

For two strains of pathogenic microorganisms - *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 - analysis of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were carried out in accordance with applicable guidelines from Clinical and Laboratory Standards Institute.

Conclusions

The results obtained under conducted experiments showed that the AEBSF can affect the growth of both tested bacterial strains in the range of tested concentrations. For α-1-antitrypsin we showed the inhibitory effect on the production of coloured substances by the strain of *Pseudomonas aeruginosa,* which are its virulence factors.

This work was partially supported by National Science Centre (**2014/15/N/NZ7/04092**).

FEMS7-3147
Pathogens / Pathogenicity - Part III

THE MECHANISMS OF THE ANTIMICROBIAL EFFECT OF ISOTHIOCYANATES ON VIBRIO CHOLERAE

K. Krause[1], D. Nowicki[1], A. Herman-Antosiewicz[2], A. Szalewska-Palasz[1]
[1]Uniwersity of Gdańsk, Department of Bacterial Molecular Genetics, Gdańsk, Poland
[2]Uniwersity of Gdańsk, Department of Biological and Medical Genetics, Gdańsk, Poland

Backgrounds

Plant secondary metabolites, as a group of compounds of natural origin, are the object of research interest due to their beneficial effects, including antimicrobial action. This is of particular importance nowadays, when the antibiotic resistance became a global medical problem. Isothiocyanates (ITC) are secondary metabolites produced by plants of the *Brassicaceae* family, of known chemopreventive and anticancer effects. We already presented their bactericidal effects against enterohaemorrhagic *Escherichia coli.*

Objectives

The main aim of our studies was the evaluation of the mechanisms of three ITCs, sulforaphane (SFN), benzyl isothiocynanate (BITC) and phenethyl isothiocyanate (PEITC) against *Vibrio cholerae*.

Methods

We used broth microdilution method to determine minimum inhibitory concentrations (MIC) and bactericidal concentrations (MBC). To assess ITCs effect on DNA/RNA synthesis, the experiment of incorporation [^{3}H]-labelled nucleotides was employed. The induction of the stringent response upon ITC treatment was evaluated using [^{32}P]-labelling and thin layer chromatography separation.

Conclusions

Our studies revealed that PEITC has the strongest antimicrobial effect against *V. cholerae,* while SFN effect was rather moderate. All ITCs inhibited significantly DNA, and to even higher extent, RNA synthesis. In concert with this observation, the exposure of the bacteria to all tested ITCs resulted in the strong induction of the stringent response and the accumulation of its alarmon, unusual nucleotide, ppGpp. This effect was reversed by the excess of particular amino acids. Our studies show strong antimicrobial potential of ITCs against *V. cholerae* which would be of great importance for designing future efficient therapy of infection by bacterial pathogens.

FEMS7-3066
Pathogens / Pathogenicity - Part III

DISRUPTION OF COTH1 AND COTH2 GENES OF MUCOR CIRCINELLOIDES BY USING A CRISPR/CAS9 SYSTEM

C. Szebenyi[1], *N. Gábor*[2], *V. Amanda*[1], *T. Eszter*[1], *K. Sándor*[1], *V. Csaba*[1], *P. Tamás*[2]
[1]*University of Szeged, Department of Microbiology, Szeged, Hungary*
[2]*MTA-SZTE "Lendület" Fungal Pathogenicity Mechanisms Research Group, Department of Microbiology, Szeged, Hungary*

Backgrounds

A CRISPR/Cas9 system has been developed and established as a robust and versatile genetic modification tool for site-specific mutagenesis of *Mucor circinelloides*. In this study, a transformation system was optimized without using plasmids to disrupt the spore cote protein H-like genes *CotH1* and *CotH2*, in *M. circinelloides*. Our transformation strategy was to use the crRNA and trans-activating crRNA (tracrRNA) together (gRNA) to guide the CRISPR-associated nuclease Cas9 to cleave double-strand breaks in the targeted DNA sequence. In parallel, deletion cassettes were constructed by PCR for the two genes and used as template DNAs for HR repair.

Objectives

M. circinelloides is one of the most studied Mucorales species and frequently used model organism in different molecular and genetic studies. Genetic modification of *Mucor* is still problematic, because the template DNA used for the transformation barely integrates in the genome and the mitotic stability of transformants is generally low.

Methods

In this study, CRISPR/Cas9 was used to disrupt the *CotH1* and *CotH2* genes in *M. circinelloides.* PEG mediated protoplast transformation was used to introduce the nuclease and the synthesized *CotH1* and *CotH2* gene specific gRNAs with the appropriate deletion cassette into the fungus.

Conclusions

Co-transformation of the Cas9 and gRNA with the deletion cassette resulted double-strand breaks of DNA, which was repaired by the own homologous recombination system of *M. circinelloides*. Molecular analysis revealed the presence of the *pyrG* gene, used as a selection marker, in the coding sequence of *CotH1* and *CotH2* genes.

The study was supported by the grants LP2016-8/2016 and GINOP-2.3.2-15-2016-00035.

FEMS7-1337
Pathogens / Pathogenicity - Part III

ANTIMICROBIAL AND INHIBITORY EFFECTS OF THERAPEUTIC-GRADE ESSENTIAL OILS AGAINST THREE ENVIRONMENTAL ISOLATED BACTERIAL STRAINS

T. Szoke-Nagy[1,2], N.E. Dina[1], S. Porav[1,2], C. Tripon[1]
[1]National Institute for Research and Development of Isotopic and Molecular Technologies, Department of Molecular and Biomolecular Physics, Cluj-Napoca, Romania
[2]Babes-Bolyai University - Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, Cluj-Napoca, Romania

Backgrounds

Evaluation of the antimicrobial effects of four essential oils against three environmental isolated bacterial strains.

Objectives

Determination of MIC, and evaluation of the antimicrobial effects for four EOs (clove, peppermint, bergamot, elemi) using disk diffusion assay.
Assessment of the bacteria structural features before and after EOs addition, using SEM.

Methods

Bacterial strains were isolated from environment by cultivation on selective media and further identified based on 16S rRNA molecular markers, using 27FB-1492R primer pair.
MIC were determined using 96-well microplates. The crude EOs were dissolved in Muller-Hinton medium with DMSO and Tween-20. Bacterial inoculum was prepared to obtain 10^6 CFU/mL bacterial suspensions. A series of two fold dilutions of each EO were prepared in each well and added the bacterial suspension. After incubation, the optical density of each well was measured at 630nm by a Microplate Reader.
An overnight culture of bacteria were adjusted to obtain 10^6 CFU/mL bacterial suspensions and spread on MH-agar medium. Paper disk with EOs was added afterwards. The inhibition zone was measured after incubation.
For SEM analysis, bacterial samples were fixed with 4% glutaraldehyde in medium for 2 hours, followed by dehydration in graded acetone series. After dehydration samples were dried on a 3 mm copper grid and coated with a 10 nm gold layer.

Conclusions

The EOs tested in this study exhibit promising antimicrobial and inhibitory effects against selected bacteria, possibly due to the presence of their bioactive constituents.

Financial support from the National Authority for Scientific Research and Innovation - ANCSI, Core Programme, Project PN16-30 02 03 is gratefully acknowledged.

FEMS7-3056
Pathogens / Pathogenicity - Part III

GENOMIC CHARACTERIZATION OF BORDETELLA PERTUSIS 0134, A VACCINE STRAIN WITH HISTORIC VALUES

K. Tadayon[1], R. Ghaderi[1], M. Noofeli[2], E. abbasi[2], P. khaki[3], M. sekhavati[1]
[1]Razi Vaccine & Serum Research Institute- Agricultural Research- Education and Ex, Aerobic Bacterial Vaccine, Karaj, Iran
[2]Razi Vaccine & Serum Research Institute- Agricultural Research- Education and Ex, Human Bacterial Vaccine, Karaj, Iran
[3]Razi Vaccine & Serum Research Institute- Agricultural Research- Education and Ex, Microbiology, Karaj, Iran

Backgrounds

The earliest serious attempts to protect Iranian citizens against pertussis were initiated in 1952 when a domestically-developed vaccine was licensed for public use.

Objectives

This study was designed and conducted to get a deeper genomic insight toward molecular characteristics of current Razi Vaccine strain of *Bordetella pertusis,* 0134.

Methods

In order to characterize the genomic properties of the exotic strain Razi Bp 0134 currently used in manufacturing the whole cell pertussis vaccine, an 8-locus multiple-locus variable-number tandem repeat analysis (MLVA) was selected. This system uses VNTR1,VNTR2,VNTR3,VNTR5,VNTR7,VNTR8,VNTR9 and VNTR10. Technically, the PCR primers and amplification cycles were designed to enable simultaneous amplification of all the 8 loci in a single round using any traditional thermocycler.

Conclusions

The sequencing-supported observations displayed that there were 8.9 copies of a 15 bp unit repeat (UR), 3.3 copies of a 12 bp UR, 7.2 copies of a 5 bp UR, 12.5 copies of a 6 bp UR, 3 copies of a 9 bp UR, 2 copies of a 12 bp UR, 5 copies of a 6 bp UR, 3.8 copies of a 9 bp UR at VNTR1,VNTR2,VNTR3,VNTR5,VNTR7,VNTR8,VNTR9 and VNTR10, respectively.
The MLVA genotyping pattern of the BP134 strain was then compared with that of the nineteen BP strains currently available at http://www.ncbi.nlm.nih.gov/genome/genomes/1008 from rest of the world. Considering the dynamic nature of Bordetella pertussis in human hosts we assume a systematicMLVA genotyping of clinical isolates is necessary to improve the currently poor epidemiological knowledge of pertussis in Iran.

FEMS7-1978
Pathogens / Pathogenicity - Part III

LEVELS OF LIPOTEICHOIC ACID (LTA), LIPOPOLYSACCHARIDE (LPS) AND MICROORGANISMS IN ROOT CANALS OF TEETH WITH PULP NECROSIS AND PERIAPICAL LESION

C.A.T. CARVALHO[1], F.E. OLIVEIRA[2], M.V.P. LEÃO[3], G. BACK BRITO[2], R. SANTANA[1], F.G. cardoso[1], M.C. VALERA[1], L.D. OLIVEIRA[2]
[1]São Paulo State University UNESP- Institute of Science and Technology, Restorative Dentistry, São Jose dos Campos, Brazil
[2]São Paulo State University UNESP- Institute of Science and Technology, Biosciences and Oral Diagnosis, São Jose dos Campos, Brazil
[3]Taubaté University, Medicine, Taubaté, Brazil

Backgrounds

Lipoteichoic acid (LTA) and lipopolysaccharide (LPS) play an important role in the etiology of pulpal and periapical alterations.

Objectives

Evaluating the levels of LTA, LPS and microorganisms in root canals of teeth with pulp necrosis and periapical lesion during endodontic treatment.

Methods

Tweenty teeth needing endodontic treatment were evaluated. The contents of canals were collected immediately after coronary opening (S1), preparation with 2.5% NaOCl (S2), use of 17% EDTA (S3) and 14 days after intracanal medication of calcium hydroxide with chlorexidine gel 2% (S4). Microorganisms were identified and quantified by Real Time PCR, LPS was quantified by chromogenic kinetic test of limulus amebocyte lysate (LAL) and LTA by enzyme-linked immunosorbent assay (ELISA). Data were analyzed by ANOVA and Tukey test ($p<0.05$). The most prevalent bacterial species in the S1 collection were *P. intermedia* (9/20), *F. nucleatum* (9/20) and *T. forsythia* (8/20). LTA and LPS were detected in 100% of the initial samples, with the highest values in samples S1 and S2. Samples S3 and S4 presented the lowest values of LTA and LPS, being statistically similar ($p>0.05$) and different from samples S1 and S2 ($p<0.05$).

Conclusions

LTA and LPS are present in 100% of the initial collections of the root canals and, during treatment, their levels only significantly reduced after the use of EDTA and intracanal medication (calcium hydroxide with chlorhexidine gel 2%). The most prevalent species were *P. intermedia*, *F. nucleatum* and *T. forsythia*.

FEMS7-3180
Pathogens / Pathogenicity - Part III

ALLELIC VARIATION, BUT NOT GENE GAIN, MAY EXPLAIN MULTI-HOST VIRULENCE IN S. DERBY ISOLATES

M. Tambassi[1], C. Bracchi[2], M. Morganti[1], S. Erika[1], L. Bolzoni[1], S. Pongolini[1], G. Casadei[1]
[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Risk Assessment Unit, Parma, Italy
[2]Università degli Studi di Parma,
Department of Chemistry- Life Sciences and Environmental Sustainability, Parma, Italy

Backgrounds

Multi-host virulence among *Salmonella* serotypes is usually attributed to specific genes while the role of Single-Nucleotide-Polymorphisms (SNPs) is not yet well understood. Data from our Regional Enteropathogens surveillance network show that the two most prevalent serotypes isolated from swine are *S.* Derby and *S.* Typhimurium monophasic variant (28.2% and 34.7%) but *S.* Derby is rarely found in human (2.6%) compared to *S.* monophasic (46.1%). *S.* Derby isolates seem therefore adapted mostly to swine with few exceptions which might have gained genetic traits responsible for multi-host virulence.

Objectives

Our work aimed to identify molecular determinants responsible for host-range broadening in S. Derby.

Methods

S. Derby isolates (n=240) were genotyped by Pulsed-Field Gel Electrophoresis (PFGE) and grouped in: A) genotypes found in isolates of swine only origin; B) genotypes found in isolates from both human and swine. We tested the virulence of 45 isolates from group A and B by invasion and replication assays in human INT-407 and swine IPEC-J2 cell lines. Group A isolates infect only IPEC-J2 cell-line while group B isolates infect both INT-407 and IPEC-J2. Whole-genome-sequencing analysis revealed 28 SNPs and 73 genes (56 chromosomal and 17 plasmidic) only present in group B isolates, but genes knockout or plasmid curing could not restrict the observed multi-host virulence. SNPs-based phylogenetic analysis showed that group A isolates cluster separately from group B, indicative of different evolutionary paths.

Conclusions

Our results suggest that multi-host virulence in S. Derby is not caused by gene gain, but more probably by SNPs allelic variation.

FEMS7-3277
Pathogens / Pathogenicity - Part III

MUSHROOM HEALTH: A SYSTEMS APPROACH

T. Taparia[1,2], M. Krijger[1], M. Hendriks[1], C. Coipan[1], W. de Boer[2], J. van der Wolf[1]
[1]Wageningen University, Biointeractions and Plant Health, Wageningen, The Netherlands
[2]Neherlands Institute of Ecology, Department of Microbial Ecology, The Netherlands

Backgrounds

Mushroom cultivation is done on a substrate of sterilized compost over which a layer of casing soil is applied. It comprises of peat, limestone and chalk, and is rich in organic matter. Fruiting of mushroom bodies relies heavily on the dynamic interactions between Agaricus bisporus and other microbes present in this casing layer. However, various Pseudomonas species present in it cause bacterial blotch on the mushrooms caps, leading to severe economic losses.

Objectives

This study aims to identify key factors determining blotch outbreaks in mushroom cultivation. It involves development of diagnostic tools to study pathogen thresholds and population dynamics in the soil. It also aims to determine suppressiveness indicators of different casing soils against these pathogens.

Methods

Taqman PCRs were developed as quantitative detection methods for various blotch pathogens. Microbiome analysis of different casing soils was performed using amplicon targeted sequencing of the 16S and ITS regions. Mushrooms cultivation bioassay were performed to check blotch prevalence under varied biotic and abiotic conditions.

Conclusions

P. gingeri is a more aggressive pathogen for bacterial blotch than P. tolaasii. Their infection and population dynamics differ significantly in casing soils. Higher microbial biodiversity in casing soil seems to lead to lower native blotch prevalence. Microbiome analysis shows that physio-chemical properties of peat, and the other raw materials used in preparation of casing affect the inherent microflora of the casing soil.

FEMS7-2650
Pathogens / Pathogenicity - Part III

IDENTIFICATION AND CHARACTERIZATION OF MYCOPLASMA FERIRUMINATORIS ISOLATES FROM ALPINE IBEXES: A NEW SPECIES VERY CLOSE TO MAJOR PATHOGENS OF DOMESTICATED RUMINANTS

C. Ambroset[1], C. Pau-Roblot[2], P. Gaurivaud[3], F. Tardy[3]
[1]VetAgro Sup, UMR Mycoplasmoses des ruminants, Marcy l'étoile, France
[2]Université de Picardie Jules Verne, Unité de Biologie des Plantes et innovation- EA 3900, Amiens, France
[3]Anses- Laboratoire de Lyon, UMR Mycoplasmoses des ruminants, Lyon, France

Backgrounds

The genus *Mycoplasma,* a group of free-living, wall-less prokaryotes gathers more than 100 species of which dozens are primary pathogens of humans and domesticated animals. Species isolated from wildlife are often understudied despite their potential role in mycoplasma overall evolutionary history. In 2013 several isolates from wild caprinae were tentatively assigned to a new species, *M. feriruminatoris*, characterized by an unusual fast growth in vitro and an important genetic proximity to ruminant pathogenic species. Recently in France, atypical isolates collected from Alpine ibexes were suspected to be *M. feriruminatoris.*

Objectives

This study was undertaken to check whether French ibexes isolates actually belong to the *M. feriruminatoris* species and further characterize them.

Methods

Phylogenetic analyses were performed to identify the isolates and explore their taxonomic position. The population diversity was characterized by genomic macrorestriction and by examining the capacity of different strains to produce capsular polysaccharides, a feature now known to vary amongst ruminant pathogenic species.

Conclusions

We reported the first isolation of *M. feriruminatoris* from Alpine ibexes in France. Phylogenetic analyses suggested that *M. feriruminatoris* might constitute the 4th species of a genetic cluster that contained so far only important ruminant pathogens. Despite their collection in a restricted region of the Alps, French isolates were not clonal, signifying an important diversity of the new species. Strains were able to produce concomitantly two types of capsular polysaccharides, β-(1→6)-galactan and β-(1→6)-glucan, with variation in their respective ratio, a feature never described so far in mycoplasmas.

FEMS7-1265
Pathogens / Pathogenicity - Part III

CHARACTERIZATION OF MOLECULAR MECHANISMS ASSOCIATED WITH FLUOROQUINOLONE RESISTANCE IN MYCOPLASMA AGALACTIAE

J. Tatay-Dualde[1], M. Prats-van der Ham[1], C. de la Fe[1], A. Paterna[1], A. Sánchez[1], J.C. Corrales[1], A. Contreras[1], Á. Gómez-Martín[1]
[1]University of Murcia, Animal Health, Espinardo Murcia, Spain

Backgrounds

M. agalactiae is the main causative agent of contagious agalactia, a disease affecting small ruminants whose importance is due to its economic impact, mainly caused by a drop in milk production. Antimicrobial treatment is the main applied control measure against this disease. Quinolones are an effective group of antimicrobials inhibiting the growth of *M. agalactiae*, but in the last years, various reports have demonstrated an increase of resistance in field isolates due to its massive use. In this context, previous studies have proved the importance of *gyrA, gyrB, parC* and *parE* genes modifying quinolone susceptibility in other mycoplasma species. Nevertheless, the molecular mechanisms involved in the acquisition of quinolones resistance in *M. agalactiae* have not been elucidated yet.

Objectives

The aim of this study was to analyze the DNA variations which affect quinolone susceptibility in *M. agalactiae*.

Methods

Two field isolates (Ag280 and Ag310) and the reference strain of *M. agalactiae*, PG2 (NCTC 10123) were selected to obtain *in vitro* resistant mutants by serial passes at subinhibitory enrofloxacin, marbofloxacin and moxofloxacin concentrations. Afterwards partial sequences of their *gyrA, gyrB, parC* and *parE* genes were analyzed.

Conclusions

Changes related to variations in quinolones susceptibility were found in *gyrB, parC* and *parE*. Besides, *parC* was the first gene showing alterations when changes in susceptibility to quinolones occurred. Thus, this gene is the most suitable target for a rapid study of quinolone resistance in field isolates of *M. agalactiae***.**

Acknowledgements

This study was funded by project AGL2013-44771-R awarded by the Spanish Ministry of Economy, government of Spain, and co-financed by FEDER funds.

FEMS7-1275
Pathogens / Pathogenicity - Part III

MOLECULAR CHARACTERIZATION OF MYCOPLASMA MYCOIDES SUBSP. CAPRI FIELD ISOLATES FROM SPAIN

J. Tatay-Dualde[1], M. Prats-van der Ham[1], C. de la Fe[1], Á. Gómez-Martín[1], A. Paterna[1], J.C. Corrales[1], A. Contreras[1], A. Sánchez[1]
[1]University of Murcia, Animal Health, Espinardo Murcia, Spain

Backgrounds

Mycoplasma mycoides subsp. capri (Mmc) is one of the main causative agents of caprine contagious agalactia. Besides, the absence of accurate control methods eases its spread between different herds within endemic areas. In this context, the possible dispersion of the agent towards different herds due to asymptomatic carriers highlights the need of molecular typing schemes which offer robust and reproducible epidemiological
information. From all the methods available nowadays, multilocus sequence typing (MLST) is the most suitable technique for genotyping bacterial isolates,
as it allows unambiguous and highly reproducible strain discrimination.

Objectives

The aim of this study was to assess the genetic variability of different strains of Mmc from a contagious agalactia endemic area through multilocus sequence typing.

Methods

For this purpose, five house-keeping genes (*fusA, glpQ, gyrB, lepA, rpoB*) from 39 field isolates were analyzed. These isolates were obtained from different geographic areas of Spain, between the years 2004 and 2015. The partial sequences of the reference strain PG3,
which are available at GenBank, were also studied.

Conclusions

Our study proved a higher
genetic variability in Mmc populations than in other contagious agalactia causing mycoplasmas, such as *M. agalactiae*. Despite the significant differences found between
the assessed field isolates, they could be classified according to their geographical origin. Moreover, it was also possible to detect genetic differences between Mmc strains coming from the same herd at the same sampling time, which may need to be taken into consideration when designing or arranging prophylactic strategies.

Acknowledgements

This study was funded by project AGL2013-44771-R awarded by the Spanish Ministry of Economy, government of Spain, and co-financed by FEDER funds.

FEMS7-0670
Pathogens / Pathogenicity - Part III

HELICOBACTER PYLORI SECRETED PROTEIN HP1286 TRIGGERS APOPTOSIS IN MACROPHAGES VIA ERK-MAPK-DEPENDENT PATHWAYS

R. Tavares[1], *S. Pathak*[1]
[1]*Stockholm University, Molecular Biosciences- The Wenner-Gren Institute, Stockholm, Sweden*

Backgrounds

Helicobacter pylori is a Gram-negative bacterium known to selectively colonize the human gastric mucosa of more than 50% of the world's population. Considering the non-invasive nature of *H. pylori*, secreted proteins are believed to play an important role in the bacterial survival and ultimately disease development.
Macrophages constitute a powerful line of defense against *H. pylori*. The final disease outcome is highly dependent on the bacterial ability to modulate the effector functions of activated macrophages.

Objectives

Investigate the role of *H. pylori* secreted protein HP1286 in the regulation of macrophage responses.

Methods

Eight different *H. pylori* strains were tested for recombinant purified HP1286 (rHP1286) expression. Primary human monocyte-derived macrophages (MDM) and macrophage cell lines (RAW 264.7, THP-1) were used for the cell assays. Binding assays and measurement of apoptosis were determine using LSRFortessa flow cytometer. Caspase 3 activation was measured using the Caspase 3/7 activity assay. Activation of TNF and ERK MAPK signalling pathways were verified by immunoblotting.

Conclusions

Exposure to rHP1286 induced apoptosis in macrophages in a time-and dose-dependent manner. Although interaction of rHP1286 was observed for several other cell types, rHP1286 failed to induce apoptosis under similar conditions, indicating a macrophage-specific effect of the protein. rHP1286 induced activation of caspase 3 and ERK MAPK signaling pathways. These results provide functional insight into the potential role of HP1286 during *H. pylori* infection. Considering the ability of HP1286 to induce macrophage apoptosis, the protein could possibly help in the bacterial escape from activated macrophages and persistence in the stomach.

FEMS7-0071
Pathogens / Pathogenicity - Part III

EFFECTS OF LEMONGRASS OIL ON MULTISPECIES ORAL BIOFILM FORMATION IN VITRO

S. Taweechaisupapong[1], W. Pinsuwan[2], W. Suwannarong[3], W. Khunkitti[4]
[1]Faculty of Dentistry- Khon Kaen University,
Biofilm Research Group and Department of Oral Diagnosis, Khon Kaen, Thailand
[2]Faculty of Dentistry- Khon Kaen University, Biofilm Research Group, Khon Kaen, Thailand
[3]Faculty of Dentistry- Khon Kaen University,
Department of Periodontology and Biofilm Research Group, Khon Kaen, Thailand
[4]Khon Kaen University, Faculty of Pharmaceutical Sciences, Khon Kaen, Thailand

Backgrounds

Cymbopogon citratus (lemongrass) oil is a volatile oil obtained from the lemongrass leaves. Lemongrass oil (LG) is mainly composed of citral, a natural mixture of geranial and neral and some myrcene, geraniol and geranyl acetate which is a potential natural biocide for use as a disinfectant. It was reported to exhibit antibacterial activity towards periodontal pathogens, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. However, there is no report on inhibitory effects of LG against subgingival biofilm formation.

Objectives

The purpose of this study was to investigate the inhibitory effects of LG on subgingival biofilm formation using an *in vitro* model.

Methods

Subgingival plaque samples from 5 periodontitis patients were cultivated in saliva-coated 96-well microtiter plates in the presence of LG at concentrations 1 - 6 µl/ml under anaerobic atmosphere at 37°C for 2, 4 and 8 days by replacing every 2 days with fresh medium alone or LG-containing medium. Biofilm formation was determined quantitatively by crystal violet staining.

Conclusions

The results showed that LG exhibited more than 70% anti-biofilm activity at 6 µl/ml. These results revealed that LG is able to inhibit *in vitro* subgingival biofilm formation and suggest potential for developing LG as a natural oral hygiene product against oral infection in people.

FEMS7-2161
Pathogens / Pathogenicity - Part III

SUBCELLULAR LOCATION OF CANDIDATE EFFECTOR PROTEINS FROM SPORISORIUM SCITAMINEUM, THE CAUSAL AGENT OF SUGARCANE SMUT

N. Teixeira-Silva[1], P. Schaker[1], S. Dinesh-Kumar[2], C. Monteiro-Vitorello[1]
[1]University Of Sao Paulo / Luiz de Queiroz College of Agriculture, Genetics Department, Piracicaba, Brazil
[2]University of California - Davis, Department of Plant Biology, Davis, USA

Backgrounds

Sugarcane is one of the most valuable crops worldwide mostly due to the great economic value of its subproducts. The production of sugarcane can be affected by many diseases including smut, caused by the biotrophic fungus *Sporisorium scitamineum*. Disease establishment is fully dependent on the environmental conditions and the sugarcane genotype, leading to the emission of a whip like structure responsible for teliospores spreading in the late stages. A previous analysis of the pathosystem using dual RNAseq-based transcriptome uncovered the expression of genes coding for candidate effectors.

Objectives

This work aimed to determine the subcellular plant compartment targeted by four *S. scitamineum* candidate effectors most expressed in early interaction using transient expression assay in *N. benthamiana*.

Methods

The coding sequence of mature protein was cloned to obtain candidate effector-green fluorescent protein (Citrine) fusions downstream of a 35S promoter in an *Agrobacterium tumefaciens* binary vector. The fusion proteins were transiently expressed by agroinfiltration and their accumulation in leaf cells was determined by confocal microscopy and immunoblots.

Conclusions

All proteins accumulated at detectable levels. Results revealed that *S. scitamineum* candidate effectors encode proteins which target various plant compartments, such as cytosol, membrane and nucleus, and some of them seems to undergo post-translational modifications. Further analysis will be performed in order to define the potential role of these proteins within host cells during disease establishment.

FEMS7-0272
Pathogens / Pathogenicity - Part III

STREPTOCOCCUS SUIS WITH HAEMOLYTIC ACTIVITY INDUCES CASPASE-1-DEPENDENT PYROPTOSIS IN HUMAN MONOCYTES

H. Thananchai[1], *J. Khikhuntod*[1], *K. Duangsonk*[1], *M. Deeudom*[1]
[1]*Faculty of Medicine - Chiang Mai University, Microbiology, Chiang Mai, Thailand*

Backgrounds

Pyroptosis is an inflammatory cell death which play an essential role in controlling intracellular infection. Recently, there are several studies reported that pore-forming toxin from gram positive bacteria can induce pyroptosis in many cell types. However, the pyroptotic induction by *Streptococcus suis* that also produce pore-forming toxin namely suilysin has not been identified.

Objectives

To investigate whether *S.suis* can induce pyroptosis in U937 human monocytic cell line.

Methods

U937 cell line was infected with nine clinical *S. suis* serotype 2 strains isolated from infected Northern Thai individuals and one reference stain from diseased pig (P1/7). Pyroptotic cells were determined by using fluorescent labeled inhibitors of caspase (FLICA)-1 flow cytometry assay.

Conclusions

We found that three live *sly*+ *S. suis* strains and P1/7 strain could activate caspase-1 and induce pyroptosis in U937 cell line. In addition, the activation of caspase 1 by *S.suis* was not resulted from K+ efflux. Although the level of suilysin production was not measured, we observed that the ability to induce pyroptosis by *S. suis* was correlated with their haemolytic activity. Taken together, our results suggested that *S. suis* with haemolytic activity can induce pyroptosis in U937 human monocytic cell line. It is likely that suilysin or other components might be involved in caspase 1 activation. Further identification of the effector mechanism that play a role in pyroptotic induction may contribute to better understanding of the pathogenesis of *S. suis* infection.

FEMS7-2117
Pathogens / Pathogenicity - Part III

COMPETITIVE FITNESS OF CLOSTRIDIUM DIFFICILE STRAINS

V. Tkalec[1], *M. Rupnik*[1,2]
[1]*National Laboratory of Health- Environment and Food, Department for Microbiology Research, Maribor, Slovenia*
[2]*University of Maribor- Faculty of Medicine, Department of Microbiology, Maribor, Slovenia*

Backgrounds

Clostridium difficile is the leading cause of nosocomial gastrointestinal infections. There are currently over 500 *C. difficile* PCR ribotypes known and some, including 014/020, are more frequently encountered than others. Common PCR ribotypes have been associated with certain phenotypic properties, however data on their competitive fitness are sparse.

Objectives

The objective of our study was to evaluate growth competition between 014/020 strains and strains of less common and rare PCR ribotypes.

Methods

Altogether five *C. difficile* strains of 014/020 (n=2), less common 002 (n=1) and rare PCR ribotypes (n=2) were included. Differences in levofloxacin minimal inhibitory concentrations (mg/L) were used to differentiate strains after co-culturing. Pairs of strains were mixed in 1:1 ratio and diluted in fresh BHIS broth. Dilution was repeated after every 24 hours for up to ten cycles. The number of viable cells for each of two strains was determined by selective plating at the end of every cycle. Competitive indices (CI) were calculated by standard methodology.

Conclusions

In this study, no apparent competitive advantage was observed for 014/020 strains in comparison to other less endemic strains. Interestingly, an oscillatory pattern in proportion of two strains was observed for some co-cultures while in one pair of strains no differences in CI were observed during first few passages, but later rare strain always outcompeted 014/020 strain.

FEMS7-0617
Pathogens / Pathogenicity - Part III

DEVELOPMENT AND VALIDATION OF A BIOLUMINESCENT BSL2 SURROGATE MODEL FOR EARLY ANTITUBERCULOSIS DRUG SCREENING

E. Torfs[1], M. Bidart de Macedo[1], F. Cools[1], B. Vanhoutte[1], L. Maes[1], G. Caljon[1], P. Delputte[1], P. Cos[1], D. Cappoen[1]
[1]University of Antwerp, Laboratory of Microbiology- Parasitology and Hygiene LMPH, Wilrijk - Antwerp, Belgium

Backgrounds

Tuberculosis (TB), an ancient infectious disease caused by *Mycobacterium tuberculosis*, still remains a major threat for global health. In 2015, there were an estimated 10.4 million new TB cases worldwide and an estimated 1.4 million people died from TB. Despite the intensive efforts to eradicate TB, there is still an urgent need for the development of novel TB drugs to address the current TB epidemic in an efficient manner. To facilitate early drug screening by avoiding the use of the highly infectious and slow-growing pathogen, a less pathogenic and faster growing surrogate model could be used instead.

Objectives

In this study, we compared different *in vitro* characteristics of a panel of seven *Mycobacterium* species and developed a luminescent reporter strain to generate a rapid and convenient surrogate model for early screening processes.

Methods

The strains were compared for their (a) *in vitro* growth, (b) *in vitro* susceptibility to a range of first- and second-line TB drugs, and (c) potential to survive and multiply inside macrophages using a RAW246.7 cell line as a surrogate for primary immune cells. Finally, a bioluminescent reporter strain was developed by electroporation of the mycobacterial strains with the pSMT1 plasmid, containing the *luxAB* genes of *Vibrio Harveyi*, for a more convenient readout of screening processes.

Conclusions

Based on the comparison of the *in vitro* characteristics of the different *Mycobacterium* species, a faster growing, bioluminescent surrogate model was developed and validated for the early screening of compounds and compound libraries with potential anti-TB activity.

FEMS7-2567
Pathogens / Pathogenicity - Part III

DISTRIBUTION OF TRIMETHOPRIM RESISTANCE GENES IN CHILEAN SHIGELLA SONNEI ISOLATES

C. TORO[1], C. García[1], L. Cádiz[1], M.T. Ulloa[1], G. Hermosilla[1], J.C. Salazar[1]
[1]Facultad de Medicina- Universidad de Chile- ICBM, Programa de Microbiología y Micología, Santiago, Chile

Backgrounds

The most common mechanism of trimethoprim (TMP)-resistance is the acquisition of dihydrofolate reductase enzyme insensitive to this drug. Previous molecular characterization of TMP-genes resistance in Chilean isolates of *S. sonnei* looking for *dfrA1*, *dfrA8* and *dfrA14*, showed solely the presence of *dfrA8* in strains isolated before 2009. However, during this year *dfrA14*-positive strains were detected, and after that between 2010-2013 *dfrA1* were detected mostly associated to class 2 integron. These genetic markers were absent in one TMP-resistance *S. sonnei* strain. Sequencing the genomic DNA of this strain allowed to identify the *dfrA15* allele.

Objectives

To identify the distribution of *dfrA1*, *dfrA8*, *dfrA14* and *drfA15* TMP-resistance genes in chilean *S. sonnei* strains isolated between 2009-2013.

Methods

Presence of TMP resistance genes was determined in 212 *S. sonnei* isolates, 28 from them were TMP-sensitive strains. Conventional PCR was conducted to detect the four TMP-resistance genes mentioned above, and class integron. Tiling PCR was used to characterize the genetic organization of class 2 integron. Clonality was analyzed by PFGE.

Conclusions

dfrA14 was present as the only TMP-resistance marker in 52% of strains, *dfrA15* was present in 33% (71/212) and 52 of them coded for *dfrA1* and *dfrA15*, meanwhile 13% do not harbour TMP resistance genes, being all of them sensitive to TMP. Strains harboured *dfrA1-dfrA15* linked to the presence of class 2 integron and most of them clustered in a nearly clonal group by PFGE. This distribution also correlated with the isolation period, showing a dynamics of trimethoprim genetic markers prevalent in Chilean *S. sonnei* strains.

FEMS7-2674
Pathogens / Pathogenicity - Part III

THE SEARCH FOR NEW TREATMENT OPTIONS AGAINST BURKHOLDERIA PSEUDOMALLEI

A. Torres[1], *B. Ross*[1], *J. Myers*[1], *G. Pradenas*[1], *L. Muruato*[1], *D. Tapia*[1]
[1]*UTMB, Microbiology and Immunology, Galveston, USA*

Backgrounds

Burkholderia pseudomallei is the causative agent of melioidosis. Aside from being a Tier 1 select agent due to its availability and ease to be aerosolized, it is a multidrug-resistant pathogen that was estimated to cause human infections and deaths worldwide. Treatment of *B. pseudomallei* usually involves long and tedious antibiotic treatment that last several weeks/months. However, even with treatment, infection relapse occurs in approximately 1 in 16 patients as *B. pseudomallei* is able of establishing a latent infection.

Objectives

Here, we used a library of 400 FDA approved compounds as part of the Pathogen Box to screen for compounds that can inhibit or kill *B. pseudomallei.*

Methods

After identifying six inhibitory compounds (Levofloxacin, Rifampicin, Auranofin, Miltefosin, and two pentaminidine analogs MMV688271 and MMV688179, we determined their MIC and MIC_{50} and determined their bacteriostatic or bactericidal properties. We also tested the ability of the bacteria to persist a supralethal dose of each compound. Further, we tested the effectiveness of the compounds in a murine model of melioidosis infection monitoring colonization and dissemination using *B. pseudomallei* K96243 strain.

Conclusions

Although the compounds did not provide as much protection as current treatments, there is evidence to suggest that improving route, dose, and solubility of MMV68827 and MMV68817 will result in more viable treatments that could help combating melioidosis infections.

FEMS7-0997
Pathogens / Pathogenicity - Part III

COMPARISON AND ENZYMATIC CHARACTERIZATION OF TYPE VD PATATIN-LIKE LIPASES

T. Trunk[1], J.P. Morth[2], D.J. Slade[3], J.C. Leo[1]
[1]University of Oslo, Department of bioscience, Oslo, Norway
[2]University of Oslo, Centre for Molecular Medicine, Oslo, Norway
[3]Virginia Polytechnic Institute and State University, Department of Biochemisttry, Blacksburg, USA

Backgrounds

Type V secretion systems and lipases of the patatin-like family have been shown to be important virulence factors in certain bacteria. *Pseudomonas aeruginosa* expresses a recently described type Vd secretion system with a C-terminal β-barrel for transmembrane transport and a N-terminal passenger domain, called patatin-like protein D (PlpD). The PlpD passenger domain displays an α/β hydrolase fold common for lipases with a catalytic Ser-Asp dyad. Once transported across the outer membrane, the passenger domain is cleaved of and forms homodimers in the extracellular space.

Objectives

The objective of this study is the characterization of the patatin-like lipase of *P.aeruginosa*, investigating the role of PlpD during *Pseudomonas* infection as well as the role of PlpD dimerization in enzyme activity and its comparison to PlpD homologues found in other bacteria (*Aeromonas hydrophila, Burkholderia pseudomallei, Fusobacterium nucleatum, Ralstonia solanacearum*, *Vibrio cholera*).

Methods

PlpDs role in the infection process will be tested via leaf infiltration assays in *Arabidopsis thaliana*, as well as in the zebrafish embryo model, employing light, fluorescence and electron microscopy. The potential direct toxic effect on mammalian cells will also be tested. The role of dimerization will be investigated using crosslinking, size exclusion chromatography and by disruption of the dimerization interface via targeted mutagenesis.

Conclusions

Characterization of the lipase and its role in infection will contribute to understanding and counteracting the pathogenesis of *Pseudomonas*. Furthermore, comparison of the activities and specificities of PlpD homologues will reveal functional differences between the lipases of the individual pathogens and contribute to understanding differential mechanisms of pathogenesis.

FEMS7-0625
Pathogens / Pathogenicity - Part III

TO DISCUSS THE VANCOMYCIN MINIMAL INHIBITORY CONCENTRATION DATA ON METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN BACTEREMIA

S.Y. Tsai[1], C. Fu-Chieh[2], L. Chon-Fu[3], L. I-Hong[1], H. Cheng-Wei[1], H. Yan-Jiun[1], C. Yi-Ting[1], K. Chien-Feng[4]
[1]Mackay Memorial Hospital, Department of Laboratory Medicine, Taipei, Taiwan R.O.C.
[2]Mackay Memorial Hospital, Infection control center, Taipei, Taiwan R.O.C.
[3]Macao Centro Hospitalar Conde de Sao Januario, Centro Hospitalar Conde de Sao Januario, Macau, Macau
[4]Mackay Memorial Hospital, Department of Infectious Disease, Taipei, Taiwan R.O.C.

Backgrounds

Vancomycin, the first line antibiotic for Methicillin-resistant Staphylococcus aureus(MRSA) bacteremia, is indicated inappropriate to administer when MIC greater than 2ug/mL, including 'susceptible' strains. Therefore, MIC should be accurately identified to avoid inappropriate antibiotic use.

Objectives

The study aims to investigate if other tests are necessary to improve the accuracy of a MIC result.

Methods

We collected 216 strains of MRSA since 2009 to 2015 and then used three methods, including auto-machine method (Vitek II), E-test and microdilution to assess the MICs of Vancomycin for the different strains of MRSA. The chi-squared test is used to determine whether there is a significant difference between the two different methods.

Conclusions

According to the MICs detected by Vitek II, 28, 95, and 93 strains had MICs of 2, 1, and 0.5 respectively. Using E-test and microdilution, only 198 strains and 195 strains showed consistent MICs, respectively. Only 15 of 28 strains, whose MIC was 2 as per Vitex II, were consistent with the results in E-test and microdilution. The proportion of MIC=2 detected from the Microdilution test was only 6.94% whereas the proportion from the Vitek II was 12.96%. The difference in proportions is significant, $\chi^2(1, N = 216) = 4.37$, $p = 0.037$. But the detection rate between E test (6.94%) and Microdlilution test(6.94%) were not statistically significant ($p = 1.00$).
Sum up, we suggest that in the setting of MRSA bacteremia, a Vancomycin MIC of 2ug/ml by Vitek II analysis should be confirmed by E-test and microdilution to avoid improper use of antibiotics.

FEMS7-3039
Pathogens / Pathogenicity - Part III

RV0089: DOES IT HAVE A ROLE IN GROWTH AND PATHOGENESIS OF M. TUBERCULOSIS?

G. Tyagi[1], P. Singh[1], R. Sinha[1], N. Sharma[1], M. Bose[1], M. Varma-Basil[1]
[1]Vallabhbhai Patel Chest Institute- University of Delhi, Microbiology, Delhi, India

Backgrounds

Biotin is an essential micronutrient required by all domains of life. In *Mycobacterium tuberculosis* it acts as a cofactor for many essential enzymes. It has been shown that exogenous biotin present in the host serum is not sufficient for bacterial growth, hence *M. tuberculosis* synthesizes biotin on its own.

Objectives

To understand the role of *Rv0089* gene in biotin biosynthesis & its effect on lipidomic profile, pathogenicity and biotin concentration in *Mycobacterium tuberculosis.*

Methods

pKO vector was used for the construction of a knockout strain *Mtb∆Rv0089* using allelic replacement method. It was grown on Sauton's medium with or without biotin for growth kinetic, stress, colony morphology & electron microscopic studies. Lipid quantification and lipidomic profiles were studied using the phosphovanillin assay and Thin Layer Chromatography (TLC) respectively. RAW 264.7 cell line was infected with *Mtb∆Rv0089* and H37RV. Immunoprecipitation was performed to quantify the biotinylated proteins in recombinant strain.

Conclusions

Deletion of *Rv0089* gene rendered the bacteria with a stationary phase growth defect and altered colony morphology. *Mtb∆Rv0089* had lower lipid content as compared to H37Rv. Addition of biotin improved the lipid composition and cell wall dynamics of *Mtb∆Rv0089*. The mutant was attenuated in macrophages and had a lower concentration of biotinylated proteins. **The results suggest that *Rv0089* gene facilitates the survival of *M. tuberculosis* during stationary phase and is required when there is no exogeneous biotin present in the medium.**

FEMS7-0635
Pathogens / Pathogenicity - Part III

SALMONELLA TYPHIMURIUM VIRULENCE FACTORS SOPB, SPTP AND PPHB CONTRIBUTE TO EVASION OF AUTOPHAGY IN THE AMOEBA DICTYOSTELIUM DISCOIDEUM AND RAW264.7 MACROPHAGES

I.M. Urrutia[1], B. Salas[1], C. Valenzuela[1], P. Fernandez[1], S.A. Álvarez[1], R. Escalante[2], C.A. Santiviago[1]
[1]Laboratorio de Microbiología- Universidad de Chile,
Departamento de Bioquímica y Biología Molecular, Santiago, Chile
[2]Instituto de Investigaciones Biomédicas "Alberto Sols" CSIC-UAM,
Departamento de Modelos Experimentales de Enfermedades Humanas, Madrid, Spain

Backgrounds

Autophagy plays a crucial role during infection acting as a defense mechanism against intracellular pathogens. As a consequence, many bacterial pathogens have developed the ability to evade this process. Autophagy is regulated by several intracellular signaling pathways, such as those linked to MAPK and PI3K/AKT/mTORC1. In these pathways, activation of MAPK and/or mTORC1 results in the repression of autophagy.

Objectives

In these work, we evaluated the contribution of *S*. Typhimurium virulence factors SopB, SptP and PphB in the evasion of autophagy in *Dictyostelium discoideum* and RAW264.7 macrophages. SopB is a phosphatidylinositol phosphatase that activates AKT, SptP has a tyrosine phosphatase domain that inactivates Erk, and PphB is a non-characterized hypothetical serine-threonine phosphatase.

Methods

We constructed mutant strains Δ*sopB*, Δ*sptP* and Δ*pphB* derived from *S*. Typhimurium 14028s, and performed infection assays using *D. discoideum* and RAW264.7 macrophages at a multiplicity of infection of 1000 bacteria/cell. After 1 h of infection, total protein fractions obtained from infected cells were subjected to western blot using antibodies against mTOR, p-mTOR and LC3 proteins, classic autophagy markers.

Conclusions

Our results in RAW264.7 suggest that virulence factors SopB and PphB contribute to *S*. Typhimurium evasion of autophagy, while SptP promotes this process. We are currently evaluating the contribution of SopB, SptP and PphB in the *S*. Typhimurium evasion of autophagosome-lysosome fusion in *D. discoideum* and RAW264.7 by confocal microscopy using cells expressing RFP-GFP-LC3 constructs.

Supported by FONDECYT grant 1140754, 1171844, MINECO grant BFU2015-64440-P and CONICYT fellowships 21150005, 22151032, 21140615, 21140692.

FEMS7-1169
Pathogens / Pathogenicity - Part III

COMPARATIVE GENOTYPING AND PHENOTYPING OF ASPERGILLUS FUMIGATUS ISOLATES FROM HUMANS, DOGS AND THE ENVIRONMENT

I. Valdes[1], J. van den Berg[1], A. Haagsman[2], N. Escobar[1], F. Hagen[3], P.J. Haas[4], J. Houbraken[5], H. Wösten[1], H. de Cock[1]
[1]Utrecht University, Microbiology, Utrecht, The Netherlands
[2]Utrecht University, Veterinary Medicine, Utrecht, The Netherlands
[3]Canisius-Wilhelmina Hospital, Medical Microbiology and Infectious Diseases, Nijmegen, The Netherlands
[4]UMC Utrecht, Medical Microbiology, Utrecht, The Netherlands
[5]CBS-KNAW Fungal Biodiversity Centre, Applied and Industrial Mycology, Utrecht, The Netherlands

Backgrounds

Aspergillus fumigatus is a ubiquitous saprotrophic fungus and an opportunistic pathogen in humans and dogs and because of its ubiquity, hundreds of *A. fumigatus* spores are inhaled on a daily basis. In most cases, the inhalation of spores is harmless but in humans, with an immunodeficiency, an invasive form of pulmonary aspergillosis (IPA) can develop which have a mortality rate of 50% or higher. *A. fumigatus* can cause also non-invasive sino-nasal aspergillosis (SNA) in immunocompetent dogs.

Objectives

Here a comparative phenotypic and genetic analysis of *A. fumigatus* isolates from canine SNA, clinical isolates from humans and environmental isolates from the air was performed to find out if inherent differences between the three groups can be found and to possibly determine what characteristics enable strains of *A. fumigatus* to infect dogs or humans.

Methods

Tandem repeats in the cyp51A gene, sequencing of calmodulin (*caM*),beta-tubulin(*benA*) genes as well as microsatellite(STR*Af*) analysis were performed in order to detect genetic differences within isolates. Results showed that that dogs with SNA are infected by one genotype only whereas human patients are infected by various genotypes.Interestingly, phenotypic analysis indicated that the isolates from dogs are most variable in growth speed and morphology as compared to human and environmental isolates. K-means clustering of the measurements of colony growth in several media showed three different clusters, one of then contain mainly *A.fumigatus* isolates from dog patients while the other two were composed by a mixture of human and environmental isolates

Conclusions

Our observation shows that canine isolates are phenotypically more diverse that their environmental and human counterparts. The basis of this heterogeneity might be due to genomic differences or epigenetic variations that occur during the infection process in dogs. Our results suggest that the canine isolates might represent a subgroup of *A. fumigatus* that are responsible for SNA.

FEMS7-1131
Pathogens / Pathogenicity - Part III

METHYLOME OF RALSTONIA SOLANACEARUM AND TRANSCRIPTOMIC CHANGES DURING POTATO INFECTION

M. Puigvert[1], N. Sánchez-Coll[1], R. Guarischi[2], I. Erill[3], A. Macho[4], J. Setubal[2], M. Valls[1]
[1]Universitat de Barcelona, CRAG, BARCELONA, Spain
[2]Universidade de Sao Paulo, Biochemistry, Sao paulo, Brazil
[3]University of Maryland Baltimore County, Biological Sciences, Baltimore, USA
[4]Shanghai Center for Plant Stress Biology, PSC, Shangai, China

Background

Ralstonia solanacearum is a ß-proteobacterium that causes bacterial wilt on important crops such as tomato and potato. *R. solanacearum* survives in soil and waterways, infects plants through the roots, migrates apoplastically and finally multiplies extensively in the xylem vessels causing plant death.

Objectives/Methods

Here, we have determined and compared the methylomes of two *R. solanacearum* strains. We have also analyzed the transciptome of strain UY031 at diffferent stages of potato colonisation.

Conclusions

Novel methylation motifs were identified, one of them associated with a DNA methylase unique to strain UY031. In addition, some virulence loci were hyper- or hypo-methylated, suggesting that methylation may modulate their expression.

We detected expression for >90% *R. solanacearum* genes both from isolated bacteria or *in silico* selected transcripts sequenced from infected plant tissues. Global transcriptional profiling provided insight into the intercellular environment encountered by this plant pathogen and the carbon and energy sources it utilizes during plant infection. Differential expression of the type three secretion system and its associated effectors was observed at various potato colonization stages. In addition, we identified several *R. solanacearum* genes that are significantly up-regulated during infection but had not been previously identified as virulence factors. This work will help identifying bacterial genes that are key for successful plant infection, which can be targets for novel antibacterial drugs.

FEMS7-2990
Pathogens / Pathogenicity - Part III

GENOMIC, METABOLIC AND IN PLANTA TRANSCRIPTOMIC COMPARISON OF THE POTATO SOFT ROT ENTEROPATHOGENS DICKEYA SOLANI AND DICKEYA DIANTHICOLA

F. Van Gijsegem[1], J. Pédron[2], D. Faure[3], Y. Raoul des Essart[4]
[1]INRA, iEES - Institute of Ecology and Environmental Sciences, PARIS, France
[2]UPMC, iEES-Institute of Ecology and Environmental Sciences of Paris, PARIS, France
[3]CNRS, Institute for Integrative Biology of the Cell - I2BC, Gif-sur-Yvette, France
[4]CNRS, Institute for Integrative Biology of the Cell - I2BC, Gif-sur-Yvette, France

Backgrounds

In potato cultures, the 4th main food crop worldwide, blackleg and soft rot caused by soft rot enterobacteria is one of the most devastating diseases. In addition to the endemic *Pectobacterium* populations, two *Dickeya* species (*D. dianthicola* and *D. solani*) emerged successively and recently in potato cultures in Europe though *Dickeya* populations were, initially, considered to be restricted to tropical and subtropical areas.

Objectives

To explore the role of the *D. dianthicola* and *D. solani* species traits in their ecological fitness and to acquire knowledge on the regulation of virulence in these pectinolytic enterobacteria, we undertook comparative genomic, metabolic and in planta transcriptomic analyses of one potato field-isolate of each species.

Methods

Definition of common and strain-specific genes was achieved using Blast analysis, metabolic capabilities using Biolog plates. Gene expression profiles after in vitro growth or tuber infection were determined by RNAseq.

Conclusions

D. solani and *D. dianthicola* genomes are highly syntenic. Both genomes only harbour a few hundred genes present in only one of the two species. These species-specific genes are often clustered in genomic regions and most of them regroup genes that are predicted to be involved in metabolism/transport. The expression of several of these species-specific genes is modulated *in planta* as compared to *in vitro* bacterial growth pointing to an involvement in plant-bacteria interactions. A differential modulation of several genes that are common to both species was also observed *in planta*. This highlights the importance of regulatory networks in the diversity of related strains/species in their interaction with their hosts.

FEMS7-1177
Pathogens / Pathogenicity - Part III

ESSENTIAL OIL AND POSTDISTILLATION WASTE OF JUNIPERUS COMMUNIS IN BIOCONTROL OF OPPORTUNISTIC PATHOGEN CANDIDA ALBICANS

B. Vasilijević[1], A. Paunkov[1], J. Knežević-Vukčević[1], D. Mitić-Ćulafić[1], S. Cvetković[1], D. Orčić[2], M. Franciškovič[2], B. Nikolić[1]
[1]University of Belgrade- Faculty of Biology, Chair of Microbiology, Belgrade, Serbia
[2]University of Novi Sad Faculty of Sciences,
Department of Chemistry- Biochemistry and Environmental Protection, Novi Sad, Serbia

Backgrounds

Candida albicans is a benign member of the gut microbiome of healthy individuals. However, the disturbance of balance in gut microbiome in immunocompromised patients can result in uncontrolled growth of *C. albicans* and lead to invasion of deeper mucosal tissue or dissemination to other organs; eventually it can enter to the bloodstream and induce systemic infections. The increasing resistance to antifungal compounds directed investigation to search for therapeutic alternatives among aromatic and medicinal plants. Among others, *Juniperus communis* is interesting for its antimicrobial properties but its anticandidial activity was only scarcely investigated.

Objectives

The aim of this study was to examine the anticandidial and antiadhesive properties of Serbian wild-growing *J. communis* var. *saxatilis* essential oil (EO) and postdistillation waste (PDW).

Methods

MIC assay on *C. albicans* ATCC10231 was used to determine MIC/MFC values, while checkerboard assay was used to determine the mode of action in binary combinations of EO/PDW with commercial fungicide bifonazole. The *in vitro* adhesion assay with EO and PDW was performed using human colon epithelial cancer HT-29 cell line.

Conclusions

MIC and MFC values of EO were determined at 12.5mg/mL, while binary combination of EO with bifonazole showed additive effect. Although effect of PDW was weaker (MIC at 25 mg/mL), in checkerboard assay it induced synergistic effect with bifonazole. In adhesion assay only EO showed notable potential to reduce pathogen colonization (72% inhibition). This preliminary work showed that *J. communis* EO and PDW could inhibit growth and adherence of *C. albicans*.

FEMS7-1935
Pathogens / Pathogenicity - Part III

ANTIFUNGAL EFFECT OF JUNIPERUS COMMUNIS ESSENTIAL OIL AND POSTDISTILLATION WASTE AGAINST SELECTED MICROMYCETES

B. Vasilijević[1], A. Paunkov[1], B. Nikolić[1], D. Orčić[2], M. Franciškovi[2], A. Džamić[3], D. Mitić-Ćulafić[1], J. Knežević-Vukčević[1], A. Ćirić[4]
[1]University of Belgrade- Faculty of Biology, Chair of Microbiology, Belgrade, Serbia
[2]University of Novi Sad Faculty of Sciences,
Department of Chemistry- Biochemistry and Environmental Protection, Novi Sad, Serbia
[3]University of Belgrade - Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Belgrade, Serbia
[4]University of Belgrade - Institute for Biological Research "Siniša Stanković",
Department of Plant Physiology, Belgrade, Serbia

Backgrounds

Taking into account the occurrence of micromycetes in the environment and food products, their potential toxicity and pathogenicity, the importance of their control is evident. Synthetic substances targeting plant fungal pathogens frequently produce toxic residues which contaminate environment and poison foodstuff and feedstuff. In addition, occurrence of fungicidal resistance to conventional antimycotics is growing. To overcome the problem biological control systems frequently based on phytochemicals are developing. Literature data indicate that *Juniperus communis* essential oil (EO) possesses antifungal capacity, but its postdistillation waste (PDW) has not been previously tested.

Objectives

The aim of this study was to compare the antifungal effect of Serbian wild-growing *Juniperus communis* var. *saxatilis* EO and PDW against *Aspergillus*, *Trichoderma* and *Penicillium* species.

Methods

The chemical composition of EO and PDW was determined using GC-MS and LC-MS/MS analyses, respectively. *In vitro* microdilution assay was used to determine the minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations.

Conclusions

Chemical analysis revealed α-pinene, sabinene and δ-cadinene as dominant constituents of EO. On the other hand rutin, quinic acid, catechin and epicatechin were abundantly presented in PDW. EO possessed only inhibitory activity with MIC of 12.5 mg/mL for all tested strains. However, antifungal potential of PDW was remarkably higher, with MIC and MFC values obtained in the ranges 0.112-0.9 mg/mL and 0.225-0.9 mg/mL, respectively. The most sensitive was *Aspergillus versicolor*. Obtained results showed high antifungal potential of PDW and direct research to its further investigation as potent antimycotic agent.

FEMS7-2985
Pathogens / Pathogenicity - Part III

CSL2, A NOVEL CHIMERIC LYSIN TO FIGHT INFECTIONS CAUSED BY THE EMERGING ZOONOTIC PATHOGEN STREPTOCOCCUS SUIS

R. Vázquez[1], M. Domenech[1], M. Menéndez[2], P. García[1]
[1]Centro de Investigaciones Biológicas CSIC, Molecular Microbiology and Infection Biology, Madrid, Spain
[2]Instituto de Química Física Rocasolano IQFR-CSIC, Química Física Biológica, Madrid, Spain

Backgrounds

Abuse and misuse of antibiotics have favored the selection and spread of multiresistant bacterial strains, leading to an alarming situation for severe infections treatment. Thus, development of alternative antimicrobial agents, such as bacteriophage –encoded endolysins (or enzybiotics), should nowadays be a priority. *Streptococcus suis* is an emerging zoonotic pathogen that infects both pigs and humans. The economic importance of swine and the severity of *S. suis* infections in humans taken together with the facts that there is no effective vaccine and multiresistant strains have already been reported, justify the development of an endolysin-based antimicrobial against *S. suis*.

Objectives

The aim of this work was the design, production and characterization of a novel chimeric endolysin specifically directed towards *S. suis*, taking advantage of our knowledge on the modular nature of bacteriophage lytic enzymes.

Methods

Chimeric enzyme Csl2 was produced in *Escherichia coli*, purified, and its activity tested *in vitro* against different *S. suis* strains and other Gram-positive bacteria. In addition, a *S. suis* biofilm model was set up for testing the ability of this enzyme to disaggregate biofilms. Finally, *in vivo* assays of Csl2 activity against *S. suis* infection were carried out in an adult zebrafish model.

Conclusions

Csl2 displayed a narrower lytic spectrum than that of its parental enzyme, focusing mainly on *S. suis* and some other streptococci from mitis group. Our new enzyme was able to both disaggregate and kill *S. suis* cells forming biofilms, and total protection of *S. suis* infected adult zebrafish was achieved by microinjecting 2 mg Csl2/kg.

FEMS7-1242
Pathogens / Pathogenicity - Part III

IDENTIFICATION OF BACTERIAL GENES INVOLVED IN THE RECOGNITION OF ESCHERICHIA COLI P4 BY BOVINE MAMMARY EPITHELIAL CELLS

M. VEDRINE[1], C. BERTHAULT[1], C. LEROUX[1], M. REPERANT[1], F.B. GILBERT[1], P. RAINARD[1], P. GERMON[1]
[1]INRA - Centre Val de Loire, 37, NOUZILLY, France

Backgrounds

Mastitis remains a major infection of dairy cows and an important issue for the dairy industry, in particular infections due to *Escherichia coli* strains. During infection, the immune response that takes place in order to eliminate infectious agent, requires recognition of bacteria by host cells, including mammary epithelial cells (MEC).
In a mouse model, infections induced by strain *E. coli* P4 show a strong colonisation of the mammary gland, while this strain has a low stimulating power on MEC.

Objectives

Our objective was to identify the genes responsible for this weak stimulation.

Methods

We screened a library of 2 000 P4 transposon mutants to identify mutants with increased ability to stimulate MEC.

Conclusions

The transposon insertion sites for thirteen mutants were identified and grouped into two classes of mutants: (i) mutants with an altered lipopolysaccharide (LPS) and (ii) others mutants which mechanism of action are at present unknown. LPS represents a highly active stimulus of the innate immune system. It has an O-polysaccharide moiety, a core oligosaccharide, and a lipid A domain. Some mutants in LPS biosynthesis genes have lost the O-polysaccharide region, while others have an altered composition. In parallel to complementation analyses of a set of selected mutants, inflammation triggered by LPS molecules with or without an altered O-antigen will be further studied in a bovine model.
This project will thus provide new knowledge about the pathways activated during the recognition of the pathogen, and which could then be modulated to improve the host response to intramammary infection by *E. coli*.

FEMS7-2238
Pathogens / Pathogenicity - Part III

ELEVATED TEMPERATURE EFFECT ON PSEUDOMONAS SYRINGAE-TOMATO PLANT INTERACTION

L. Scalschi[1], E. Fernandez-Crespo[1], M. Pitarch[1], A. Gonnzález-Hernandez[1], E. Llorens[1], L. lapeña[1], P. García-Austín[1], G. Camañes[1], B. Vicedo[1]
[1]Universitat Jaume I, Ciències Agràries i del Medi Natural, Castellón de la Plana, Spain

Backgrounds

Nowadays climate change is having numerous impacts on plants. Simultaneously this change can also have direct effects on many plant pathogens therefore affecting pathogen development and survival rates and modifying host susceptibility, resulting in changes in the impact of diseases on crops.Understanding how plants and pathogens respond to this change is essential to conserve the former ones.

Objectives

One of the components of climate change are the elevated temperatures. In order to understand how this component affects both plant and pathogen response as well as their interaction,

Methods

Studies were carried out using the Pseudomonas syringae-tomato pathosystem. Two experimental conditions were compared. The first one consists in growing and inoculating the plants at 26 ° C while for the second one plants were grown at 31 °C and further inoculated with a bacteria acclimatized at 31 °C.

Conclusions

Subsequently, both plant and bacterial response was analyzed, demonstrating that, under the later conditions, the plant acclimatizes faster than the bacteria. The results showed that elevated temperatures not only reduced the infection by 50%, but also modified the infection phenotype since greater chlorosis and less necrosis was observed in these plants. This was accompanied by a significant reduction in the number of colony forming units. Our study also revealed that plants are able to adapt to elevated temperatures by undergoing physiological changes such as increases in chlorophyll content and photosynthetic rate.

In view of these results, plant response as well as bacterial response to increased temperatures are being characterized at both transcriptomic and metabolomic level.

FEMS7-1625
Pathogens / Pathogenicity - Part III

INITIAL EVALUATION OF THE ROLE OF MUCR IN VIRULENCE AND OUTER MEMBRANE PROPERTIES OF BRUCELLA OVIS PA

R.S. Sidhu-Muñoz[1], R. Losa-Rodríguez[2], N. Vizcaíno[1]
[1]Edificio Departamental and IBSAL. Universidad de Salamanca, Dpto. Microbiología y Genética, Salamanca, Spain
[2]Edificio Departamental. Universidad de Salamanca, Dpto. Microbiología y Genética, Salamanca, Spain

Backgrounds

Brucella ovis provokes contagious epididymitis in rams and consequently important economic loses in the livestock sector. The virulence of this rough *Brucella* (without O-polysaccharide chains in the lipopolysaccharide) has been scarcely studied when compared to that of smooth *Brucella* (bearing O-polysaccharide). Additionally, there is no specific vaccine for *B. ovis* infection and heterologous *B. melitensis* Rev1, considered the best available vaccine against *B. ovis*, displays several important drawbacks.

Objectives

The aim of this work was the construction and initial characterization the Δ*mucR* mutant of *B. ovis* PA. MucR was previously found to be involved in cell envelope modifications and to be necessary for the virulence of smooth *B. melitensis*.

Methods

mucR was deleted by homologous recombination with a plasmid bearing the gene inactivated by overlapping PCR. Characterization of *B. ovis* Δ*mucR* included growth characteristics, outer membrane-related properties and intracellular behaviour in professional and nonprofessional phagocytes.

Conclusions

Compared to the parental strain, the Δ*mucR* mutant showed delayed growth in solid and liquid rich medium and a smaller colony size. It exhibited autoagglutination ability in static liquid culture medium and higher susceptibility in a disc assay to sodium deoxycholate and sodium dodecyl sulphate. Internalization of Δ*mucR* in HeLa cells and J744 murine macrophages was similar to that of the parental strain. However, its intracellular survival over time was impaired in a similar way to that of an attenuated mutant in the type IV secretion system. These results encourage the evaluation of the virulence and vaccine properties of *B. ovis* PA Δ*mucR* in animals.

FEMS7-2559
Pathogens / Pathogenicity - Part III

INITIAL CHARACTERIZATION OF THE BRUCELLA OVIS PA ΔZNTR MUTANT DEFECTIVE IN A TRANSCRIPTIONAL REGULATOR INVOLVED IN ZINC HOMEOSTASIS

R.S. Sidhu-Muñoz[1], R. Losa-Rodríguez[2], N. Vizcaíno[1]
[1]Edificio Departamental and IBSAL. Universidad de Salamanca, Dpto. Microbiología y Genética, Salamanca, Spain
[2]Edificio Departamental. Universidad de Salamanca, Dpto. Microbiología y Genética, Salamanca, Spain

Backgrounds

Brucella ovis causes ram contagious epididymitis, an infectious disease for which specific vaccines are not available. Previous works with *B. abortus* identified *zntR* as a gene involved in zinc homeostasis and required to maintain a chronic infection in mice. Deletion of *zntR* in *B. ovis* would allow to analyse its role in virulence and led to the development of a specific recombinant attenuated vaccine.

Objectives

In this work we describe the construction of the Δ*zntR* mutant of *B. ovis* PA and its initial evaluation aiming to determine the role of ZntR in zinc homeostasis and virulence of *B. ovis* PA.

Methods

zntR was deleted by homologous recombination with a plasmid bearing the gene inactivated by overlapping PCR. Characterization of *B. ovis* Δ*zntR* included growth characteristics, susceptibility to $ZnCl_2$ and a zinc chelator, outer membrane-related properties and intracellular behaviour in professional and nonprofessional phagocytes.

Conclusions

Growth of *B. ovis* PA and its derived Δ*zntR* mutant in solid an liquid rich medium was similar. Addition of a zinc chelator to the culture medium abolished growth of Δ*zntR* and *B. ovis* PA but it was restored in both strains after addition of $ZnCl_2$. Susceptibility to compounds conventionally used to evaluate outer membrane properties was not modified by *zntR* deletion. Internalization and survival in murine macrophages and HeLa cells of the Δ*zntR* mutant and *B. ovis* PA were also similar. However, according to the results described for *B. abortus* Δ*zntR*, evaluation of the *B. ovis* mutant in mice must be performed before discarding its lack of attenuation.

FEMS7-3044
Pathogens / Pathogenicity - Part III

VISIBLE AND UVA LIGHT AS A POTENTIAL MEANS OF PREVENTING ESCHERICHIA COLI BIOFILM FORMATION IN URINE AND ON MATERIALS USED IN URETHRAL CATHETERS

T. Vollmerhausen[1], A. Conneely[2], C. Bennett[2], V. Wagner[3], J. Victor[4], C. O'Byrne[1]
[1]National University of Ireland Galway, Bacterial Stress Response Group Microbiology, Galway, Ireland
[2]National University of Ireland Galway, National Centre for Laser Applications, Galway, Ireland
[3]Teleflex, Cambridge, Cambridge, USA
[4]Teleflex, Reading, Reading, USA

Backgrounds

Catheter-associated urinary tract infections are the most common hospital-acquired infection, for which *Escherichia coli* is the leading cause.

Objectives

This study investigated the efficacy of 385 nm and 420 nm light for inactivation of *E. coli* attached to the silicone matrix of a urinary catheter.

Methods

Using urine mucin media, inactivation of planktonic bacteria and biofilm formation was monitored using silicone coupons.

Conclusions

Continuous irradiance with both 385 nm and 420 nm wavelengths with starting cell density population 10^3 CFU ml^{-1} reduced planktonic suspensions of *E. coli* to below the detection level after 2h and 6h, respectively. Bacterial attachment to silicone was successfully prevented during the same treatment. Inactivation by 385 nm and 420 nm was found to be dependent on media, cell density and oxygen, with less inhibition on planktonic suspensions when higher starting cell densities were used. In contrast to planktonic suspensions in PBS, continuous irradiance of pre-established biofilms showed a greater reduction in survival compared to urine mucin media after 24 hours. Enhanced inhibition for 385 nm and 420 nm light in urine mucin media was associated with increased production of reactive oxygen species. These findings suggest 385 nm and 420 nm light as a promising antimicrobial technology for the prevention of biofilm formation on urethral catheters.

FEMS7-1440
Pathogens / Pathogenicity - Part III

MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN KALININGRAD, RUSSIAN EXCLAVE ON BALTIC SEA

A. Vyazovaya[1], N. Solovieva[2], G. Akhmedova[3], E. Turkin[3], A. Gerasimova[1], O. Narvskaya[1], V. Zhuravlev[2], I. Mokrousov[1]
[1]St. Petersburg Pasteur Institute, Laboratory of Molecular Epidemiology and Evolutionary Genetics, St. Petersburg, Russia
[2]Research Institute of Phthisiopulmonology, Department of Etiological Diagnostics, St. Petersburg, Russia
[3]Regional Anti-Tuberculosis Dispensary, Bacteriology Laboratory, Kaliningrad, Russia

Backgrounds

Kaliningrad (former Koenigsberg) is the westernmost Russian region and makes an exclave on the Baltic Sea separated from mainland Russia by Lithuania and Poland. Despite some decline in tuberculosis (TB) incidence (50.6/100000 in 2015), the rate of primary multidrug resistance (MDR) in the last five years increased from 23.9% to 31.8%.

Objectives

We aimed to assess current structure of *Mycobacterium tuberculosis* population in the Russian/EU borderline Kaliningrad region and see how the situation has changed since our previous study in 2006.

Methods

The 93 *M. tuberculosis* isolates were recovered from newly-diagnosed pulmonary TB patients in 2015. Drug susceptibility testing was done by absolute concentrations method. DNA was subjected to spoligotyping; spoligotypes were compared to SITVIT_WEB. LAM isolates was detected by testing specific *Rv0129c* SNP and were further tested for RD-Rio, RD115, LAM-RUS markers. Beijing B0/W148 cluster was identified by detection of *Rv2664-Rv2665*::IS*6110*.

Conclusions

Most of isolates were drug resistant (32.3% pansusceptible, 51.6% MDR). Beijing genotype predominated in the collection (61/93). Russian epidemic clone Beijing B0/W148 was detected in 22/93 isolates and was mainly MDR (21/22). Non-Beijing families included T, LAM, Ural, S, and X (32 isolates in total) and presented 16 spoligotypes. *M. tuberculosis* population in Kaliningrad region is dominated by the Beijing (65.6%), T (14%) and LAM (12%) families. The MDR-associated Beijing genotype increased its rate in the last 10 years from 44% to 65%; its circulation continues to critically influence the adverse epidemiological situation of MDR-TB here and bears an impact on the EU neighbors.
Funding: Russian Science Foundation grant #14-14-00292.

FEMS7-0698
Pathogens / Pathogenicity - Part III

CHARACTERISTIC OF LOW-1 MOLECULAR-MASS PENICILLIN-BINDING PROTEINS, NAGZ AND AMPR IN AMPC B-LACTAMASE EXPRESSING YERSINIA ENTEROCOLITICA

X. WANG[1], *H. Jing*[2]
[1]*National Institute for Communicable Disease Control and Prevention - China CDC, Beijing, China*
[2]*National Institute for Communicable Disease Control and Prevention- China CDC, Laboratory of Emergency, Beijing, China*

Backgrounds

Yersinia enterocolitica encodes a chromosomal AmpC β-lactamase under the regulation of the classical *ampR-ampC* system.

Objectives

To obtain a further understanding of the regulation characteristics of the AmpC expression of *Y. enterocolitica*. Elucidate the characteristics of LMM PBPs (PBP4, PBP5a, PBP5b and PBP7), NagZ and AmpR in the regulation of *ampC* in *Y. enterocolitica*.

Methods

We investigate the roles of low-molecular-mass penicillin-binding proteins (LMM PBPs) in the expression of AmpC β-lactamase by monitoring the ampC promoter activity in a series of LMM PBP mutants. PBP5b is involved in the process of *ampC* regulation, and the effects of PBP4, PBP5a and PBP7 were relatively low. Additionally, the quadruple deletion strain YEΔ4Δ5aΔ5bΔ7 (*pbp4, pbp5a, pbp5b* and *pbp7* inactivated) possessed the highest ampC promoter activity. Then, we explored the role of NagZ in *ampC* regulation and found that nagZ inactivation caused two completely different results in YEΔD123 (*ampD1, ampD2* and *ampD3* inactivated) and YEΔ4Δ5aΔ5bΔ7, indicating that two *ampC* regulation pathways exist in *Y. enterocolitica*.

Conclusions

We found that NagZ is essential for AmpC hyperproduction through the Δ*pbps* pathway, while in the Δ*ampD* pathway, NagZ was displaced by an unknown enzyme which could not hydrolyze the p-nitrophenyl-β-Nacetyl-D-glucosaminide. The inactivation of AmpR reduced the *ampC* expression in both YEΔD123 and YEΔ4Δ5aΔ5bΔ7, indicating that AmpR is necessary in the AmpC hyperproduction in these two pathways.

FEMS7-1745
Pathogens / Pathogenicity - Part III

IN DEPTH ANALYSIS OF STREPTOCOCCUS PYOGENES COLONIZATION AND INVASION OF A HUMAN TISSUE

A. Weckel[1,2,3], C. Mehats[4,5,6], T. Guilbert[7,8,9], M. Longo[1,2,3], C. Poyart[1,2,3,10,11], A. Fouet[1,2,3,11]
[1]Institut Cochin INSERM U1016, Barriers and pathogens, Paris, France
[2]CNRS UMR8104, Barriers and pathogens, Paris, France
[3]Université Paris Descartes, Barriers and pathogens, Paris, France
[4]Institut Cochin INSERM U1016,
GENOMICS- EPIGNENETICS AND PHYSIOPATHOLOGY OF REPRODUCTION, Paris, France
[5]Université Paris Descartes,
GENOMICS- EPIGNENETICS AND PHYSIOPATHOLOGY OF REPRODUCTION, Paris, France
[6]CNRS UMR8104, GENOMICS- EPIGNENETICS AND PHYSIOPATHOLOGY OF REPRODUCTION, Paris, France
[7]Institut Cochin INSERM U1016, Imag'ic facility, Paris, France
[8]CNRS UMR8104, Imag'ic facility, Paris, France
[9]Université Paris Descartes, Imag'ic facility, Paris, France
[10]Assistance Publique Hôpitaux de Paris,
Service de Bactériologie- Groupe Hospitalier Paris Centre Cochin-Hôtel Dieu-Broca, Paris, France
[11]Centre National de Référence des Streptocoques, Hôpital Cochin, Paris, France

Backgrounds

The strictly human pathogen *Streptococcus pyogenes*, also known as Group A Streptococcus (GAS), is a Gram-positive bacterium responsible for non-invasive and invasive diseases, including endometritis, and post streptococcal sequelae, leading altogether to 517,000 deaths yearly. Postpartum endometritis refers to infection of the decidua and is responsible for puerperal sepsis, a leading cause of peripartum maternal death. GAS presents various virulence factor repertoires and *emm28* strains are associated with endometritis. Models recapitulating early stages of tissue invasion are lacking.

Objectives

To set up an *ex vivo* model of human tissue GAS colonization and invasion, to decipher the cellular and molecular mechanisms involved in GAS invasive infections.

Methods

The decidua contains uterine stromal cells, derived from maternal endometrial cells, and resident immune cells. Human decidual samples are obtained from cesarean delivery at term of an uncomplicated pregnancy. These samples are infected *ex vivo* with an *emm28* GAS fluorescent relevant clinical isolate and mutant strains. Infection is monitored by confocal microscopy to follow the time-course of bacterial colonization and invasion of the tissue. Thorough image analysis is performed to precisely localize bacteria within the tissue and to quantify invasion kinetic and breadth. Cytotoxicity of the different strains is measured by analyzing nuclear damages.

Conclusions

Within the first four hours, GAS forms biofilm-like structures at the surface of the decidua with an extended colonization surface. In contrast to mutant strains, the wild-type strain kills a notable proportion of cells. This non-motile bacterium penetrates several micrometers deep into the tissue in a previously undescribed manner.

FEMS7-1737
Pathogens / Pathogenicity - Part III

CONDITIONALLY PATHOGENIC GUT MICROBES PROMOTE LARVAL GROWTH BY INCREASING REDOX-DEPENDENT FAT STORAGE IN HIGH SUGAR DIET-FED DROSOPHILA

T.W. Whon[1], N.R. Shin[1], M.J. Jung[1], D.W. Hyun[1], H.S. Kim[1], P.S. Kim[1], J.W. Bae[1]
[1]Kyung Hee University,
Department of Life and Nanopharmaceutical Sciences and Department of Biology, SEOUL, Republic of Korea

Backgrounds

Changes in the composition of the gut microbiota contribute to the development of obesity and subsequent complications associated with metabolic syndrome. However, the role of increased numbers of certain bacterial species during the progress of obesity and factor(s) controlling the community structure of gut microbiota remain unclear.

Objectives

The present study aimed to identify the inter-relationship between *Drosophila melanogaster* and their resident gut microbiota under chronic high sugar diet (HSD) conditions.

Methods

Chronic feeding of a HSD to *Drosophila* resulted in a predominance of resident uracil-secreting bacteria in the gut. Axenic insects mono-associated with uracil-secreting bacteria or supplemented with uracil under HSD conditions promoted larval development. Redox signaling induced by bacterial uracil promoted larval growth by regulating sugar and lipid metabolism via activation of p38a mitogen-activated protein kinase.

Conclusions

The present study identified a new redox-dependent mechanism by which uracil-secreting bacteria (previously regarded as opportunistic pathobionts) protect the host from metabolic perturbation under chronic HSD conditions. These results illustrate how *Drosophila* and gut microbes form a symbiotic relationship under stress conditions, and changes in the gut microbiota play an important role in alleviating deleterious diet-derived effects such as hyperglycemia.

FEMS7-0671
Pathogens / Pathogenicity - Part III

TRIGGERING THE ACTIVITY OF THE H2-T6SS OF PSEUDOMONAS AERUGINOSA IN VITRO AND CHARACTERISATION OF A COGNATE EFFECTOR

T.E. Wood[1], L.P. Allsopp[1], S. Wettstädt[1], A. Förster[2], P.S. Freemont[2], A. Filloux[1]
[1]Imperial College London, Life Sciences, London, United Kingdom
[2]Imperial College London, Medicine, London, United Kingdom

Backgrounds

The type VI secretion system (T6SS) is an important virulence determinant of many pathogenic Gram-negative bacteria and is often also vital in inter-bacterial competition. *Pseudomonas aeruginosa* PAO1 encodes three such systems, of which the H2- and H3-T6SS contribute to bacterial fitness during infection.

Objectives

In this work we sought to establish expression conditions in which the H2-T6SS could be studied *in vitro*, and probe the roles of effector proteins linked with this system.

Methods

We present conditions for detection of a functional H2-T6SS and associated effector proteins by western blot.

Conclusions

We characterise one such effector and demonstrate its role in *P. aeruginosa* colonisation and infection. Bioinformatics analyses reveal that this effector is a member of a toxin superfamily commonly associated with type VI secretion systems throughout the proteobacterial phylum. Finally, we present the crystal structure of this effector and identify a fold not previously found in T6SS effector proteins.

FEMS7-1228
Pathogens / Pathogenicity - Part III

IDENTIFICATION AND CHARACTERIZATION OF NOVEL ADHESINS FROM A FISH PATHOGEN, YERSINIA RUCKERI.

A. Wróbel[1], *C. Ottoni*[1], *J. Leo*[1], *D. Linke*[1]
[1]*University of Oslo, Department of Biosciences, Oslo, Norway*

Backgrounds

Yersinia ruckeri is a Gram-negative, rod-shaped bacterium belonging to the genus *Yersinia*. It is the causative agent of enteric redmouth disease affecting salmonids globally and causing devastating economic losses in aquaculture industry.

Objectives

The aim of the research is to characterize *Y. ruckeri* inverse autotransporter adhesins for structural and functional studies. However, we encountered problems in using PCR to amplify one of the genes named ilm (invasin-like molecule)

Methods

The *Y. ruckeri* genome of the strain ATCC29473 was re-sequenced in the PacBio platform and annotated with Prokka. The Single-Molecule Real-Time (SMRT) sequencing of the PacBio platform makes is possible to overcome the limitations of other sequencing technologies - based on shorter read data - in defining highly-repeated DNA regions. We identified two inverse autotransporter adhesins that we called invasin (Inv) and (Ilm). We found that Ilm consists of 19 identical repeats with a low GC content region. In addition, the ilm gene is flanked by insertase, suggesting recent acquisition by horizontal gene transfer. Based on the new genome information, additional analyses were performed including biofilm formation assay and gene expression analysis.

Conclusions

Identification and characterization of the adhesins give valuable information on the evolution of the pathogen. Moreover, current next-generation sequencing technologies, such as SMART sequencing is a useful and an ideal approach for the sequencing of such repetitive regions. The knowledge gained through this study could also be used for the development of novel inhibitors and specific detection methods of this pathogen. Understanding the adhesion mechanism will help in characterization of the infection process.

FEMS7-1472
Pathogens / Pathogenicity - Part III

LENTIVIRUS-MEDIATED GALECTIN-3 GENE DELIVERY AMELIORATES INFLUENZA PATHOGENESIS VIA INDUCTION OF M2 MACROPHAGE ACTIVATION

C.L. Wu[1], Y. Mei-Lin[2], C. Nai-Hui[2], S. Ai-Li[2]
[1]National Chen Kung University Medical College, Department of Biochemistry and Molecular Biology, Tainan, Taiwan R.O.C.
[2]National Chen Kung University Medical College, Department of Microbiology and Immunology, Tainan, Taiwan R.O.C.

Backgrounds

Factors which are implicated in high morbidity and mortality from influenza A virus (IAV) infection include robust cytokine production (cytokine storm), excessive inflammatory infiltrates, and virus-induced lung tissue destruction. Galectin-3 belongs to a family of evolutionally conserved glycan-binding proteins with multifunctional properties. It is expressed in a variety of immune cell types during microbial invasion. It can promote migration of monocytes/macrophages and drive alternative macrophage activation. The status of macrophage polarization upon virus infection has been studied, but much remains obscure.

Objectives

We investigated the roles of galectin-3 and M2 macrophage polarization in influenza virus infection.

Methods

We used lentiviral vectors encoding mouse galectin-3 (LV-gal-3) to overexpress galectin-3 in the lung of mice, and investigated its role in influenza infection. M2 macrophage polarization was examined by immunocytochemistry, and cytokines in the BAL fluid were detected by ELISA.

Conclusions

We show that the levels of galectin-3 were increased in the lungs of mice during influenza virus infection. Galectin-3 is mainly produced by macrophages. Mice treated with LV-gal-3 attracted more inflammatory cell infiltration to the lung, especially M2 macrophages. Furthermore, lentivirus-mediated galectin-3 gene delivery protected mice against lethal influenza virus challenge by reducing inflammation and ameliorating lung injury. Furthermore, IL-10 production in the respiratory tract of mice receiving LV-gal-3 was higher than that of mice receiving the control vector LV-null.

Our results demonstrate that galectin-3 can ameliorate influenza pathogenesis by promoting M2 macrophage activation. This study provides a proof of principle for the potential therapeutic application of galectin-3 for influenza.

FEMS7-0791
Pathogens / Pathogenicity - Part III

THE ANTIMICROBIAL PEPTIDES EXERT ANTIFUNGAL ACTIVITY THROUGH IMPAIRMENT OF MITOCHONDRIA FUNCTION

Y. Xue[1], *G. Lin*[1], *C. Lan*[1]
[1]*National Tsing Hua University, Institute of Molecular and Cellular Biology, Hsinchu, Taiwan R.O.C.*

Backgrounds

Candida albicans is an important human fungal pathogen, which can cause life-threatening infections particularly in immunocompromised patients. Moreover, drug resistance in *C*. *albicans* has also emerged as a serious problem due to the widespread use of fungicides. Therefore, developing new anti-fungal drugs and studying their mechanisms of actions are very important.

Objectives

In this study, we investigated the mechanisms of several histatin 5-derived antimicrobial peptides (AMPs) involved in eradicating *Candida albicans* cells.

Methods

To study the mechanisms of the histatin 5-derived peptides, a mutant library of *C*. *albicans* was used. The susceptibility of mutants to the AMPs was examined and the results indicated a number of mutants exhibiting resistance to the AMPs testing were defective in mitochondria. These results raise a possibility that the mitochondria may be a target for the AMPs testing. Therefore, the ROS level and the oxygen consumption after peptide treatment were measured. Finally, the ROS scavenger was introduced to verify the role of ROS derived from the AMPs in *C*. *albicans* killing.

Conclusions

The AMPs testing increase the intracellular ROS levels of *C*. *albicans*. The oxygen consumption rate of the cell is also significantly decreased after the treatment with AMPs. These results indicate the peptides exert their fungalcidal activity through attacking the mitochondria.

FEMS7-1524
Pathogens / Pathogenicity - Part III

PROPER CONTROL OF FLAGELLAR ROTATION OF OPPORTUNISTIC PATHOGENS IS REQUIRED TO EVADE INNATE IMMUNITY OF HEALTHY HOSTS

K. Yamazaki[1], T. Kashimoto[1], T. Kado[1], Y. Akeda[2], T. Kodama[3], K. Yoshioka[1], S. Ueno[1]
[1]Kitasato Univ. School of Veterinary Medicine, Veterinary Medicine, Towada, Japan
[2]Univ. Hospital- Osaka Univ., medicine, Suita, Japan
[3]RIMD- Osaka Univ., Bacterial Infections, Suita, Japan

Backgrounds

Opportunistic pathogens can cause fulminant wound infection that potentially progress to necrotizing fasciitis for a short time even in healthy individuals. *Vibrio vulnificus* is one of those pathogens: 80% of the wound-infected people were not having underlying diseases and survived even in being suffered severe local symptoms, whereas 20% of the wound-infected people were having some chronic diseases and died due to fatal sepsis. However, the mechanism by which opportunistic pathogens evade immunity of healthy hosts at the site of wound infection has remained unknown.

Objectives

We explore the essential factors of opportunistic pathogens to cause wound infection, and elucidate the role of the factor in immune evasion at the site of wound infection.

Methods

We identified the proper control of flagellar rotation as an essential function for *V. vulnificus* wound infection by applying signature-tagged transposon mutagenesis to a murine wound infection model. Role of the proper control of flagellar rotation was investigated by in vivo imaging system, measuring bacterial burdens, detection of serum biomarkers of muscular damage, histopathological studies, and comparison of lethality among the routes of s.c., i.m., and i.v. inoculation. Furthermore, we used neutropenic mice to investigate the relationship between opportunistic pathogens and neutrophils.

Conclusions

We demonstrated that the proper control of flagellar rotation contribute to spread of bacteria through subcutaneous tissue and to invade muscular tissue from dermis in wound infection, and the invasion of the muscular tissue is a key process for evasion of neutrophil killing and for proliferation of bacteria in wound infection.

FEMS7-0716
Pathogens / Pathogenicity - Part III

EVALUATION OF THE IMMUNOGENICITY AND VACCINE POTENTIAL OF RECOMBINANT ACINETOBACTER BAUMANNII GLYCOPROTEIN A1S_0556

C.Y. Yang[1], Y.C. Kung[1], S.C. Kuo[2]
[1]National Chung Hsing University, Institute of Molecular Biology, Taichung, Taiwan R.O.C.
[2]National Health Research Institutes, National Institute of Infectious Diseases and Vaccinology, Miaoli, Taiwan R.O.C.

Backgrounds

Acinetobacter baumannii, an opportunistic human pathogen, is a key source of nosocomial infection causing severe clinical diseases in people with compromised immune systems. It poses a great threat for human health because these organisms are resistant to most antibiotics. Vaccine is one plausible approach for prevention of multi-drug resistant bacteria.

Objectives

A general O-linked protein glycosylation system appears to be present in all clinical *A. baumannii* isolates suggesting an important role of glycoproteins in *A. baumannii* virulence. Thus, the *A. baumannii* glycoproteins may be good vaccine candidates. A1S_0556 was previously identified as an O-glycosylated protein in the membrane extracts of *A. bumannii*. In this study, we investigate whether A1S_0556 protein can be used as a vaccine antigen.

Methods

The coding regions of A1S_0556 were PCR amplified from *A. baumannii* ATCC 17978 and expressed in *E. coli*. The recombinant proteins were purified using the Ni^{2+} affinity column and used to immunize mice in the presence or absence of Freund's adjuvants. The expression of A1S_0556 in *A. baumannii* was monitored by Western blotting. The potential use of A1S_0556 as a vaccine candidate was evaluated by *in vivo* active protection assay and *in vitro* serum bactericidal assay.

Conclusions

A1S_0556 protein is ubiquitously expressed in *A. baumannii*. Mice immunized with the *E. coli* expressed non-glycosylated A1S_0556 were protected from *A. buamannii* in a pulmonary challenge model and the antisera raised against the recombinant protein with or without the presence of adjuvant possessed *in vitro* bactericidal activity. Collectively, we conclude that A1S_0556 is an ideal vaccine candidate.

FEMS7-0323
Pathogens / Pathogenicity - Part III

FIMZ OF SALMONELLA ENTERICA SEROVAR TYPHIMURIUM MEDIATES DIFFERENT PHYSIOLOGICAL FUNCTIONS

K.S. Yeh[1], C.H. Chang[1], H.C. Lin[1]
[1]National Taiwan University, Veterinary Medicine, Taipei, Taiwan R.O.C.

Backgrounds

Salmonella enterica serovar Typhimurium produces type 1 fimbriae on the outer membrane and such hair-like appendages are involved in adherence of the bacteria to host cells and tissues. The *fim* gene cluster is responsible for the phenotypic expression of type 1 fimbriae in *S*. Typhimurium. Production of type 1 fimbriae is cooperatively regulated by *fimZ*, *fimY*, *stm0551*, *fimW*, and *fimU* within the *fim* gene cluster. FimZ belongs to the response regulator of the two-component regulatory system in bacteria and has been shown to activate type 1 fimbrial expression.

Objectives

Our objective is to further explore the role that FimZ may participate besides type 1 fimbrial regulation.

Methods

A *fimZ* mutant in LB5010 strain was constructed by allelic exchange and was found to be non-fimbriate. Transcriptomic analysis by microarray revealed that the stress response related gene *cpxP*, *soxS* and type 1 fimbriae related genes *fimA-fimF*, and *fimW* were down-regulated, whereas plasmid-encoded fimbriae, chemotaxis, flagella associated genes and virulence genes like *virK*, *invC* and *mgtC* were up-regulated in the *fimZ* mutant strain. Oxidative stress response assay was performed with disc diffusion method and indicated that the *fimZ* mutant was more sensitive to H_2O_2 and paraquat than its parental strain LB5010. However, a homologue of *fimZ* from *Enterobacter cloacae* cloned in pACYC184 could not complement the *fimZ* mutant in the oxidative stress response.

Conclusions

FimZ protein of *S*. Typhimurium may serve as a multi-functional regulator that interacts with other genes in addition to the *fim* genes. Mechanisms of this crosstalk warrant further investigation.

FEMS7-1363
Pathogens / Pathogenicity - Part III

DETECTION OF MYCOPLASMA GALLISEPTICUM INFECTION FROM CHICKEN BREEDER FLOCKS AND EMBRYONATED EGGS BY REAL-TIME PCR, CULTURE AND SEROLOGY

Ö. Yılmaz[1], S. Kahya[1], K.T. Carlı[1]
[1]Institute of Health Sciences, Microbiology, Bursa, Turkey

Backgrounds

Mycoplasma gallisepticum (MG) infections cause severe economic losses in poultry industry. Because it is transmitted vertically, control of MG has become important. Upper respiratory problems caused by MG is diagnosed by detection of spesicif DNA, isolation of MG or detection of antibodies.

Objectives

This study aimed to identification of MG by using ELISA, culture and real-time PCR (rPCR) from breeder flocks, chicks and embryonated eggs. We compared this rPCR test with serology and culture for the detection of MG.

Methods

Culture and rPCR were applied to 630 egg yolk swab samples and 110 tracheal swab samples (pooled in groups of 5). 900 blood samples from breeder flocks suffering from respiratory disease problems were tested by ELISA. Swabs were serial diluted up to 10^{-4} into Frey's broth and incubated. Dilutions which is turned into orange-red, were immediately cultivated in Mycoplasma agar and incubated. At the end of the incubation 'fried egg' appearance which is occured in plates was observed with stereomicroscope. At the same time egg yolk and tracheal swabs were vortexed with Frey's broth for rPCR. Then swabs were removed and rPCR was applied to the suspensions.

Conclusions

25 (16.8%) of the pooled 148 swab samples came out positive by using MG culture, 65 (43.9%) of the same samples came out positive by rPCR. The ELISA result of 900 blood samples were 460 MG seropositive. In conclusion, rPCR is a highly specific, sensitive method for MG identification. MG infection remains a common respiratory tract infection in breeders and it continues vertically transmission.

FEMS7-0520
Pathogens / Pathogenicity - Part III

DIFFERENT TRANSCRIPTIONAL RESPONSES IN HUMAN MACROPHGE CELLS, THP-1 CELLS, TO BRUCELLA ABORTUS MUTANTS

S. Soo Jin[1], I. Young Bin[1], Y. Han Sang[1]
[1]College of Veterinary Medicine - Seoul National Univer, Department of Infectious Diseases, Seoul, Republic of Korea

Backgrounds

Brucella abortus, a zoonotic agent, causes undulant fever, arthritis, endocarditis in human and abortion and infertility in cattle. *B. abortus* is a facultative intracellular bacterium that can survive and replicate within host macrophages. Recognition of the interaction of the bacterium with host cells is crucial to elucidation of the infectious process. As the first step, *B. abortus* mutants were generated using transposon mutagenesis. Biological properties of the mutants were characterized in the growth rate, internalization and intracellular survival and replication.

Objectives

To demonstrate roles of the *B. abortus* genes, THP-1 cells were infected with *B. abortus* wild type and mutant strains C1, C10, C27, and C32, and then different transcriptional responses of the cells were determined using microarray.

Methods

After infection of THP-1 cells with *B. abortus* wildtype and mutant strains, altered gene expression was categorized by molecular function and biological process. Amount of cytokines was measured in the cell culture supernatans by ELISA.

Conclusions

Mutants C27 and C32 up-regulated the gene expression in the cells while gene expression was suppressed with mutants C1 and C10. Also, gene showed higher fold changes were listed. In the analysis of KEGG pathway, genes related with cytokine-cytokine receptor interaction, chemokine signaling pathway, and Toll-like receptor signaling pathway were down-regulated in the THP-1 cells with mutants C1 and C10. But, those genes were up-regulated with mutants C27 and C32. The alterations were coincided with the amounts of cytokines in the supernatant of the cells. This research was supported by KHIDI, the Ministry of Health & Welfare, (HI16C2130) and BK21, ROK.

FEMS7-3258
Pathogens / Pathogenicity - Part III

ATYPICAL PROTEINS EXPOSED ON THE SURFACE OF CANDIDA GLABRATA BIND TO HUMAN ENDOTHELIAL CELLS

D. Zając[1], O. Bochenska[1], A. Kozik[1]
[1]Jagiellonian University,
UJ Faculty of Biochemistry- Biophysics and Biotechnology- Department of Analytical Biochemistry, Krakow, Poland

Backgrounds

Candida glabrata is the second most common cause of fungal infections in humans after *C. albicans*. The knowledge on proteinaceous components of *C. glabrata* cell wall is still unsatisfactory, possibly suggesting that this fungus has evolved different mechanisms for the interaction with the host cells, in comparison to *C. albicans*.

Objectives

To identify proteins exposed on the surface of *C. glabrata* and involved in the interaction with human umbilical vein endothelial cells (HUVECs).

Methods

A mixture of *C. glabrata* cell wall-associated proteins was released with beta-1,6-glucanase. Using the chemical cross-linking and liquid chromatography-coupled tandem mass spectrometry methods, we identified several cell wall proteins involved in the interaction with HUVECs. The “cell surface shaving”, based on a short treatment of fungal cells with trypsin, was used to analyze the changes in *C. glabrata* cell wall proteome after co-culture with HUVECs.

Conclusions

In the present study we identified several fungal proteins that are capable of interacting with human endothelial cells. Among them, after using both cross-linking and “cell surface shaving” methods, we found "atypical" cell wall proteins that originate from the cytoplasm, such as glyceraldehyde-3-phosphate dehydrogenase 3 (Tdh3). Interestingly, alcohol dehydrogenase 1 (Adh1) and phosphoglucose isomerase (Pgi) were found to be exposed on the surface of *C. glabrata* only after the contact with endothelial cells. Our data indicate that *C. glabrata* cell wall proteins can be taken into consideration in attempts to develop new antifungal therapies.

This work was financially supported by the FBBB funds for young scientists (KNOW BMN grant no. 15/2016 awarded to D.Z.).

FEMS7-1384
Pathogens / Pathogenicity - Part III

THE ROLE OF SALMONELLA PATHOGENICITY ISLAND (SPI)1 EFFECTOR MOLECULES FOR ENTEROCYTE INVASION, INTRACELLULAR PROLIFERATION AND TRANSCRIPTIONAL ACTIVATION IN VIVO

K. Zhang[1], A. Riba[1], N. Torow[1], U. Repnik[2], G. Griffiths[2], M. Hensel[3], M. Hornef[1]
[1]Uniklinik RWTH Aachen, Institute of Medical Microbiology, Aachen, Germany
[2]University of Oslo, Department of Biosciences, Oslo, Norway
[3]University of Osnabrück, Division of Microbiology, Osnabrück, Germany

Backgrounds

Due to the lack of a suitable animal model, the molecular mechanisms of *Salmonella enterica* in epithelial cells invasion and mucosal barrier penetration have been studied using immortalized cell culture models. Recently we have established a neonatal murine infection model that allows the analysis of the contribution of both *Salmonella* and host factors during the host-microbial interplay *in vivo*.Using this model, we demonstrate spontaneous intestinal colonization, formation of intraepithelial microcolonies, mucosal translocation and spread to systemic organs following oral administration of *Salmonella*. In contrast to the situation in adult animals, mucosal translocation in the neonate intestine in the absence of M cells depends on pathogenicity island (SPI)1-mediated enterocyte invasion. Innate immune stimulation via Toll-like receptor 4 and 9 occurs in an invasion-dependent manner and induces a strong transcriptional epithelial response.

Objectives

The role of Salmonella SPI1 effector molecules for enterocyte invasion, intracellular proliferation and transcriptional activation will be studied in the established neonatal murine infection model.

Methods

In order to study single SPI1 effector molecule *in vivo*, the quadruple, triple and single mutant bacteria in combination with complementation of individual SPI1effectors are orally fed to new born mice.

Conclusions

We identify the dominant role of SipA and SopE/E_2 in enterocyte invasion, innate immune stimulation and mucosal translocation. Intraepithelial *Salmonella* microcolony formation requires fully SPI1. Together, our new mouse infection model illustrates the critical role of enterocyte invasion for mucosal translocation, innate immune stimulation and characterizes the contribution of individual SPI1 effector molecules during early infection *in vivo*.

FEMS7-2024
Pathogens / Pathogenicity - Part III

THE INTRODUCTION OF AN ACTIVE CARBONIC ANHYDRASE ALLOWS CO2-DEPENDENT BRUCELLA STRAINS TO GROW UNDER ATMOSPHERIC CO2 CONCENTRATION

L. Pérez-Etayo[1], M.J. De Miguel[2], R. Conde-Álvarez[1], P.M. Muñoz[2], I. Moriyón[1], M. Iriarte[1], A. Zúñiga-Ripa[1]
[1]Instituto de Salud Tropical. Universidad de Navarra, Microbiología y Parasitología, Pamplona, Spain
[2]Centro de Investigación y Tecnología Agroalimentaria, Unidad de Sanidad Animal, Zaragoza, Spain

Backgrounds

Brucellosis is an important zoonosis caused by bacteria of the genus *Brucella*. Animal vaccination is the main way to prevent this disease but there is no vaccine against *B. ovis* and protection is achieved using *B. melitensis* Rev1. Stopping vaccination with Rev1 when *B. melitensis* is eradicated leads to an increase in the number of infections caused by *B. ovis* and thus, research on specific vaccines is essential. Nevertheless, one of the main difficulties to develop a *B. ovis*-vaccine is the requirement of a high-CO_2 atmosphere to grow.

Objectives

To analyze the mechanisms underlying CO_2 dependence in *Brucella* and to obtain a CO_2-independent *B. ovis* strain.

Methods

We first sequenced and analyzed the genes encoding carbonic anhydrases (CAs) I and II from *B. ovis* and two *B. abortus* strains (292 and 544). Then, we inserted the genes encoding CAI and/or CAII into the genome of the three strains using the mini-Tn7 system. We studied their growth under atmospheric conditions and their infection kinetics in mice.

Conclusions

In the case of *B. ovis*, both genes encoding CAI and CAII are disrupted. In contrast, although CAI is conserved in the two *B. abortus* strains, the lack of a functional CAII seems to be responsible for the requirement of a high-CO_2 atmosphere. Consequently, the introduction of an active CAII allows *Brucella* growth under atmospheric conditions, while the role of CAI remains to be unveiled. Interestingly, the introduction of a functional CAII into *B. ovis* does not affect the virulence of this strain, and therefore, it is an excellent background for the development of specific vaccines.

FEMS7-0446
Pathogens / Pathogenicity - Part III

INFECTION RATES OF WOLBACHIA SP., BARTONELLA SP. AND RICKETTSIA SP. IN DIFFERENT POPULATIONS OF FLEAS

A. ZURITA CARRASCO[1], *R. CALLEJÓN FERNÁNDEZ*[1], *M. DE ROJAS ÁLVAREZ*[1], *C. CUTILLAS BARRIOS*[1]
[1]*University of Sevilla, Department of Microbiology and Parasitology- Faculty of Pharmacy, Sevilla, Spain*

Backgrounds

Flea-borne organisms are widely distributed throughout the world in endemic disease foci, where components of the enzootic cycle are present. However, flea-borne human pathogens have emerged recently (e.g., *Bartonella henselae*, *Rickettsia felis*), suggesting that much remains to be learned about the potential role of fleas as disease vectors. Thus flea-borne diseases could re-emerge in epidemic form because of changes in the vector–host ecology due to environmental and human behaviour modifications. On the other hand, *Wolbachia* strains are globally distributed, and currently these bacteria are considered the most abundant endosymbionts found in invertebrates.

Objectives

In the present study, we carried out a molecular detection of *Bartonella* sp. *Rickettsia* sp. and *Wolbachia* sp. in different populations of fleas isolated from different hosts from different geographical areas of Europe.

Methods

Molecular studies were performed by Polymerase Chain Reaction (PCR) of ribosomal DNA (16S, 16S-23 intergenic spacer region and 23S–5S intergenic spacer region) of *Wolbachia* sp., *Bartonella* sp. and *Rickettsia* sp., respectively.

Conclusions

In the present work, we detected the presence of *Bartonella* sp., *Rickettsia* sp. and *Wolbachia* sp. in different flea species. Some species such as *Nosopsyllus barbarus* were tested for these pathogens for the first time. Our results strongly support the role of *W. pipientis* as a common endosymbiont of fleas as well as the idea that several flea species might play an important role as vectors in human *Bartonella* sp. and *Rickettsia* sp infections in different geographical areas.

FEMS7-0273
Physiology / Biochemistry / Molecular Microbiology

THE RESPIRATORY COMMENSAL BACTERIA CORYNEBACTERIUM PSEUDODIPHTHERITICUM 090104 IMPROVES RESPIRATORY ANTIVIRAL INNATE IMMUNITY

A.K.M.H. Kober[1], P. Clua[2], A. Tada[1], G. Vizoso -Pinto[3], K. Paulraj[1], C. Rodriguez[2], H. Iida[1], S. Salva[2], S. Alvarez[2], F. Raya -Tonietti[3], V. Melnikov[4], H. Kitazawa[1], V. Julio[2]
[1]TOHOKU UNIVERSITY, Graduate School of Agricultural Science, Sendai, Japan
[2]CENTRO DE REFERENCIA PARA LACTOBACILOS CERELA-CONICET, Laboratory of Immunobiotechnology, San Miguel de Tucumán, Argentina
[3]National University of Tucuman, INSIBIO UNT-CONICET, San Miguel de Tucumán, Argentina
[4]International Science and Technology Centre, International Science and Technology Centre, Moscow, Russia

Backgrounds

The nasal administration of *Corynebacterium pseudodiphtheriticum* strain 090104 increases the elimination of *Staphylococcus aureus* from the nasal cavity in volunteers. The ability of the 090104 strain to improve respiratory immunity or its capacity to increase resistance against respiratory virus were not investigated before.

Objectives

Therefore, this work examined the effect of viable (Cb) or heat-killed (HkCb) *C. pseudodiphtheriticum* 090104 on the innate respiratory antiviral immune response and the resistance against influenza infection in mice.

Methods

Adult Balb/c mice were treated with 10^8 cells of Cb or HkCb by the nasal route during two consecutive days. Treated and untreated control mice were then nasally challenged with influenza virus. Lung tissue damage and respiratory and systemic immune responses were studied at several time points after viral challenge.

Conclusions

Cb treatment protected mice by reducing pulmonary injury and lung viral loads. This effect was associated to a differential regulation of the production of antiviral factors since respiratory levels of IFNs were enhanced ($p<0.01$) in Cb-mice. In addition, Cb down-regulated the expression of inflammatory cytokines while increased the regulatory cytokine IL-10 ($p<0.05$). Cb treatment also improved activation of lung antigen presenting cells and the numbers of IFN-γ and IL-10 producing $CD4^+$ lymphocytes. HkCb did not induce significant effects in the immune response to influenza infection, indicating that the viability of the respiratory commensal is a key factor to achieve the protective effect. These results strongly suggest that administration of Cb may represent an interesting alternative to modulate respiratory immune response and reduce respiratory virus-associated pulmonary damage.

FEMS7-0274
Physiology / Biochemistry / Molecular Microbiology

EFFECT OF IMMUNOBIOTIC BIFIDOBACTERIA ON THE INNATE ANTIVIRAL IMMUNE RESPONSE IN BOVINE INTESTINAL EPITHELIAL CELLS: IMMUNOTRANSCRIPTOMIC ANALYSIS

L. Albarracín[1], A.K.M.H. Kober[2], H. Kobayashi[2], N. Sato[2], K. Paulraj[2], W. Ikeda-Ohtsubo[2], T. Nochi[2], H. Aso[2], N. Iwabuchi[3], J.Z. Xiao[3], T. Saito[2], J. Villena[1], H. Kitazawa[2]
[1]CENTRO DE REFERENCIA PARA LACTOBACILOS CERELA-CONICET, Laboratory of Immunobiotechnology, San Miguel de Tucumán, Argentina
[2]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan
[3]Morinaga Milk Industry Co.- Ltd., Food Science and Technology Institute, Kanagawa, Japan

Backgrounds

Previous studies showed that *Bifidobacterium infantis* strain or *B. breve* strain significantly reduced rotavirus titers in infected bovine intestinal epithelial (BIE) cells.

Objectives

In this work, transcriptomic studies in BIE cells were performed to advance in the understanding the mechanisms involved in the antiviral activity of bifidobacteria.

Methods

BIE cells were stimulated with *B. infantis* or *B. breve* (5 x 10^8 cells/ml) and then challenged with the TLR3 agonist poly(I:C). BIE cells with no bifidobacteria treatment were used as controls. The transcriptomic response was evaluated 12 h after poly(I:C) challenge using the Bovine (G3) Gene Expression Microarray (Agilent). Variations in transcript abundance with a t-test $p<0.05$, and a cutoff in abundance of at least 2-fold were considered statistically different. Selected genes were further evaluated by RT-PCR.

Conclusions

TLR3 activation in BIE cells significantly increased the expression of type I interferons, interferon-induced factors and pro-inflammatory cytokines and chemokines. Both bifidobacteria improved the expression of *IFN-α* and *IFN-β* as well as the antiviral factors *RNASEL*, *MX1,* and *MX2* when compared to controls. In addition, bifidobacteria increased the expression of *IL-1β*, *IL-20*, *IL-6, CXCL2, CXCL3, CXCL6, CCL20*, and *CCL4*. Increased expression of *IL-1α* and *NLRP3* genes was also observed in *B. breve*-treated BIE cells. These results indicate that both immunobiotic bifidobacteria are able to improve the antiviral state of BIE cells that correlate with their capacity to reduce rotavirus replication. In addition, the transcriptomic analysis showed that both immunobiotic bacteria would be able to improve the recruitment and activation of immune cells to the site of viral infection.

FEMS7-0233
Physiology / Biochemistry / Molecular Microbiology

MECHANISTIC AND PHENOTYPIC CHARACTERISATION OF RGG/SHP QUORUM SENSING SYSTEM IN STREPTOCOCCUS PNEUMONIAE

I. Abdullah[1], R. Wallis[2], P. Andrew[1], H. Yesilkaya[2]
[1]University of Leicester, Infection- Immunity and Inflammation, Leicester, United Kingdom
[2]University of Leicester, Infection-Immunity and Inflammation, Leicester, United Kingdom

Backgrounds

The Rgg (Regulator Gene of Glucosyltransferase) regulators with their short signaling peptides called SHP (short hydrophobic peptides) form part of a quorum sensing system in Gram positive bacteria. They play an important role in stress response, sugar metabolism, and virulence, but their function and mechanism of action remain unclear in the important human pathogen *Streptococcus pneumoniae.* The pneumococcal type 2 D39 strain has five homologues of Rggs, and two of them (SPD_0144 and SPD_0939) are predicted to be associated with putative *shp* genes encoding for SHP peptides, which regulate their own expression, and are predicted to be required for Rgg activation

Objectives

This study was designed to identify the optimum length of SHP144 to stimulate Rgg144-mediated transcription, and quantify the functional importance of each selected SHP144 residue for Rgg144 activation and binding, and establish their importance in Rgg144's phenotypic manifestation

Methods

Several synthetic SHP144 peptides representing the C-terminal end of SHP144 were synthesised to identify active SHP144 using reporter strains P*shp144*::*lacZ*-wt and P*shp144*::*lacZ*-Δ*shp144* ('P'-promoter). Site directed mutagenesis was used for substitution of selected residues of SHP144 with alanine, and the effect of each amino acid replacement was studied using a transcriptional reporter assay. The phenotypic impacts of mutations were determined by H_2O_2 resistance, and by growth assays in chemically defined medium supplemented with different sugars

Conclusions

The SHP144 peptide regulates its own expression and the12 and 13 amino acid long synthetic peptides representing the C-terminal end of SHP144 (C12 and C13, respectively)are sufficient to stimulate P*shp144*::*lacZ* both in wild type and mutant *shp144* background. Furthermore, most of alanine replaced residues abolish P*shp144* driven *lacZ* activity, indicating the importance of selected residues in transcriptional activation.
Finally, we found that mutations of selected SHP144 residues decrease pneumococcal resistance to H_2O_2, and diminish its growth in CDM supplemented with mannose

FEMS7-1331
Physiology / Biochemistry / Molecular Microbiology

IN SILICO ANALYSIS OF SAFETY ASPECTS OF A POTENTIALLY PROBIOTIC LACTOBACILLUS PENTOSUS MP-10 ISOLATED FROM BRINES OF NATURALLY FERMENTED ALORENA GREEN TABLE OLIVES

H. Abriouel[1], B. Pérez Montoro[1], M.D.C. Casado Muñoz[1], C.W. Knapp[2], A. Gálvez[1], N. Benomar[1]
[1]University of Jaen, Health Sciences, Jaen, Spain
[2]University of Strathclyde, Department of Civil and Environmental Engineering, Glasgow, United Kingdom

Backgrounds

The genome sequence of a potential probiotic *L. pentosus* MP-10 is considered the largest genome among lactobacilli up to date highlighting its ecological flexibility and adaptability.

Objectives

Our objective was to determine the safety aspects of this bacterium since this is of great importance with regards to the future application of *L. pentosus* MP-10 as probiotic.

Methods

In this study, the genome sequence of *L. pentosus* MP-10 was annotated and analyzed *in silico* for the presence of antibiotic resistance determinants and mobile genetic elements.

Conclusions

The results obtained by means of bioinformatic tools showed the presence of different genes encoding for antibiotic resistance being highly represented by efflux pump genes. Furthermore, analysis of *L. pentosus* MP-10 genome sequence by means of ResFinder (acquired antimicrobial Resistance gene Finder) software revealed the absence of acquired antibiotic resistance genes by horizontal gene transfer. On the other hand, screening for mobile genetic elements that play a key role in the spread of antibiotic resistance determined that *L. pentosus* MP-10 genome possessed 5 prophages (two are intact, the other two are incomplete and the last one is questionable) and several transposases similar to other lactobacilli, however transposons were absent. In conclusion, *L. pentosus* MP-10 could be considered as safe since no acquired antibiotic resistance genes were found and that most of the resistance genes corresponded to efflux pump genes. Furthermore, the mobile genetic elements detected may play a role in genome diversification and evolution of this strain, and thus increase its adaptation potential.

FEMS7-3071
Physiology / Biochemistry / Molecular Microbiology

REGULATION OF SPORULATION-INHIBITED SECRETION IN ASPERGILLUS NIGER

D. Aerts[1], R.A. Ohm[1], S.G. van den Bergh[1], M. Arentshorst[2], W.R. Teertstra[1], C. Phippen[3], K.F. Nielsen[3], A.F.J. Ram[2], J.C. Frisvad[3], H.A.B. Wösten[1]
[1]Utrecht University, Biologie, Utrecht, The Netherlands
[2]Leiden University, Molecular Microbiology and Biotechnology, Leiden, The Netherlands
[3]Technical University of Denmark, Systems Biology, Lyngby, Denmark

Backgrounds

Sporulation inhibits enzyme secretion in the industrial cell factory *Aspergillus niger*. By inactivating gene *flbA*, sporulation is abolished while secretion takes place throughout the colony, including the zones of the mycelium that otherwise would sporulate. Deletion of *flbA* also impacts other processes such as cell wall thickness and cell lysis.

Objectives

The aim of this project is to identify transcription factors downstream of FlbA that control secretion.

Methods

FlbA controls (in)directly 38 transcription factor genes. So far, 4 of these genes have been analyzed. Two of them do not function in secretion but are involved in stress resistance and fumonisin biosynthesis, respectively. Two transcription factors do play a role in secretion. Inactivation of one of these genes resulted in a different secretion pattern, while in the other case less protein was secreted.

Conclusions

Together, this implies that we have identified one potential modulator and one activator linking sporulation and protein secretion. Currently, we are studying the impact of these transcription factors on gene expression and the secretome. These findings are interesting leads for improving A. niger as a cell factory.

FEMS7-1292
Physiology / Biochemistry / Molecular Microbiology

EXPRESSION AND REGULATION OF THE VIRULENCE GENE SRFJ OF SALMONELLA ENTERICA

J. Aguilera Herce[1], *A. Schikora*[2], *F. Ramos-Morales*[1]
[1]*Universidad de Sevilla, Departamento de Genética, Sevilla, Spain*
[2]*Julius Kühn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany*

Backgrounds

Salmonella is a Gram-negative facultative intracellular pathogen, which belongs to the family Enterobacteriaceae. Its pathogenic properties are partially a consequence of virulence genes that encode effector proteins. SrfJ is one of the effectors secreted by the type III secretion system (T3SS) encoded by *Salmonella* pathogenicity island 2 (SPI2) when *Salmonella* is inside the host cell. *srfJ* expression depends on the two-component system regulator SsrA-SsrB, the main regulator of SPI2, and therefore it is coordinated with the expression of the SPI2 T3SS. Interestingly, the expression of *srfJ* is induced by *myo*-inositol. This is due to the location of this gene in the *myo*-inositol utilization island, controlled by the repressor IolR.

Objectives

To understand this dual regulation system and its expression *in vitro* and *in vivo* during mammalian cell infection and plant colonization.

Methods

Fusions with *luxCDABE* operon of *Photorhabdus luminescens*. Expression measurements during animal cell infection and plant colonization.

Conclusions

The expression of *srfJ* is under the control of two promoters that are induced in different conditions. The first one is the promoter of the gene *iolE*, located upstream of *srfJ* and whose expression is regulated by IolR. The second is *srfJ* own proximal promoter region, whose main regulator is SsrB. The regulation has been studied in detail using strains with mutated regulators that affect the expression of different chromosomal virulence regions. *srfJ* is expressed from the proximal promoter during mammalian cell infection and from the *iolE* promoter during growth in plant media and during plant colonization.

FEMS7-0426
Physiology / Biochemistry / Molecular Microbiology

COMPLETE GENOME SEQUENCE AND COMPARATIVE GENOME ANALYSIS OF BACILLUS SUBTILIS SUBSP. SUBTILIS KCTC 3135(T)

S. Ahn[1,2,3], *S. Kim*[2,3]
[1]*Seoul National University, Biomedical Sciences, Seoul, Republic of Korea*
[2]*Korea Research Institute of Chemical Technology, Convergent Research Center for Emerging Virus Infection, Daejeon, Republic of Korea*
[3]*Korea Research Institute of Standards and Science, Center for Bioanalysis, Daejeon, Republic of Korea*

Backgrounds

Bacillus subtilis is one of the most ubiquitous and diverse Gram-positive bacteria, which is broadly adapted to grow in many environments especially in the soil and water sources. Previously, only draft genome sequence was available for the *B. subtilis* subsp. *subtilis* type strain.

Objectives

Here, we deeply sequenced and annotated complete genome of *B. subtilis* subsp. *subtilis* KCTC 3135^T, replacing previous draft genome.

Methods

The genomic DNA was extracted from broth culture of the strain and sequenced by Illumina sequencer. The size of the genome is approximately 4 megabase pairs with G+C ratio of 43.51%. Gene prediction was carried out using Prodigal then gene annotation was performed with various tools including NCBI prokaryotic genome annotation pipeline. The genome of *B. subtilis* KCTC 3135^T contains 4,315 CDSs, 10 rRNA operons and 86 tRNA genes. Comparative genomic analysis of *B. subtilis* subsp. *subtilis* KCTC 3135^T with 15 other *B. subtilis* subsp. *subtilis* strains and 2 *B. subtilis* subsp. *spizizenii* strains (including type strain; TU-B-10^T) was carried out using several tools. Phylogenetic trees of polC, rpoB, gyrA and ANI (Average Nucleotide Identity) distinguished *B. subtilis* subsp. *subtilis* strains from *B. subtilis* subsp. *spizizenii* strains. The *tar* genes and *tag* genes which are involved in cell wall phenotype were investigated whether various *B. subtilis* subspecies strains can be grouped based on the cell wall related genes.

Conclusions

In conclusion, the genome of *B. subtilis* KCTC 3135^T was completely sequenced and closely related *B. subtilis* subspecies can be clearly distinguished based on the genotypic comparison.

FEMS7-2403
Physiology / Biochemistry / Molecular Microbiology

RECOMBINASES ARE ESSENTIAL FOR BACTERIOPHAGE REPLICATION

M.M. Neamah[1], *J.R. Penades*[1]
[1]*University of Glasgow, Biomedical Research Centre, Glasgow, United Kingdom*

Backgrounds

DNA-single strand annealing proteins (SSAPs) are a type of recombinases frequently encoded in the genome of many bacteriophages. Since these proteins can promote homologous recombination among DNA substrates with an important degree of divergence, it has been assumed that the main role for these enzymes is to generate mosaicisms, driving phage evolution.

Objectives

Here, analysing Sak and Sak4 as representatives of two different families of SSAPs present in phages infecting the clinically relevant bacterium *Staphylococcus aureus*, we demonstrate for the first time that these enzymes are absolutely required for phage reproduction.

Methods

Deletion of the genes encoding these enzymes significantly reduced phage replication and the generation of infectious particles. Complementation studies revealed that these enzymes are required both in the donor (after prophage induction) and in the recipient strain (for infection). Moreover, our results also indicated that to perform their function, the SSAPs require the activity of their cognate single strand binding (Ssb) proteins. Mutational studies demonstrated that the Ssb proteins are also required for phage replication, both in the donor and the recipient strain.

Conclusions

In summary, our results expand the functions attributed to the SSAPs, and demonstrate that both SSAPs and SSB proteins are essential for the life cycle of the staphylococcal phages.

FEMS7-2966
Physiology / Biochemistry / Molecular Microbiology

GENOMIC CLUSTERING OF MAL GENES IN YEASTS

T. Alamäe[1], *K. Viigand*[1], *K. Põšnograjeva*[1]
[1]*University of Tartu, Institute of Molecular and Cell Biology- Department of Genetics, Tartu, Estonia*

Backgrounds

Genomic clustering of functionally linked genes is rare in eukaryotes. Yet, subtelomeric regions of *Saccharomyces cerevisiae* harbor gene families including yeast MALT (transporter), MALS (maltase or isomaltase) and MALR (regulator) genes. Similar MAL loci have been described also in *Pichia stipitis*. We have described a genomic MAL locus of a phylogenetically 'old' methylotrophic yeast *Ogataea* (*Hansenula*) *polymorpha*. This locus includes MAL1 gene encoding for maltase-isomaltase [1], and MAL2 encoding for alpha-glucoside transporter. Both proteins have extended substrate specificity. The MAL locus of *O. polymorpha* also contains two putative MAL-activator genes.

Objectives

The aim was to analyze clustering of MAL genes in phylogenetically diverse yeasts.

Methods

Gene and protein sequences were withdrawn from GenBank and UniProtKB/Swiss-Prot, the genomes were accessed mostly through Mycocosm website (http://genome.jgi.doe.gov/programs/fungi/index.jsf). Phylogenetic tree of maltase proteins [1] was built at http://phylogeny.lirmm.fr. Genomes of yeasts from different branches of the phylogenetic tree were analysed for genomic neighborhood of maltase/isomaltase/alpha-glucosidase genes. Genes potentially encoding alpha-glucoside transporters and transcriptional activators were searched adjacent to these genes to locate putative genomic loci for the utilization of alpha-glucosidic sugars.

Conclusions

No MAL loci were found in yeasts *Schizosaccharomyces pombe* and *Blastobotrys adeninivorans* which are considered 'ancient' yeasts. One putative MAL locus was found in *Lodderomyces elongisporus* and *Debaryomyces hansenii*. Several putative alpha-glucosidases and MAL loci were detected in *Lipomyces starkeyi*, *Pichia guillermondii* and *Pichia stipitis*.

Acknowledgements: This work was financed by ERC grant GLTMR1050P (PUT1050).

References

[1] Viigand K., Visnapuu T., Mardo, K. Aasamets A., Alamäe T. 2016. *Yeast.* DOI: 10.1002/yea.3157.

FEMS7-0717
Physiology / Biochemistry / Molecular Microbiology

DISSECTION OF A NOVEL METAL RESISTANCE MECHANISM IN CUPRIAVIDUS METALLIDURANS

M.M. Ali[1], K. Mijnendonckx[1], N. Leys[1], P. Monsieurs[1], D. Charlier[2], R. Van Houdt[1]
[1]SCK•CEN, Microbiology, Mol, Belgium
[2]Vrije Universiteit Brussel, Microbiology, Brussels, Belgium

Backgrounds

Cupriavidus metallidurans strains contain many genes involved in the resistance and processing of heavy metals, which are very well studied and characterized. In addition, studies at SCK•CEN underscored the rapid evolution of *C. metallidurans* strains towards significantly increased metal resistance, particularly for silver.

Objectives

Our objective is to understand the molecular mechanisms of this increased resistance to silver.

Methods

C. metallidurans strains including type strain CH34, its plasmidless derivative AE104 and NA4, isolated from a silver-sanitized drinking water system, were grown in toxic levels of silver to obtain silver-resistant mutants. The latter were characterized in detail via re-sequencing, oligonucleotide microarrays, and deletion and complementation analyses.

Conclusions

Our data indicate that *C. metallidurans* is able to adapt rapidly to toxic silver concentrations. Silver-resistant mutants were obtained from different strains, even from strains cured from the main mega-plasmid encoded metal resistance determinants, indicating that the canonical efflux mechanisms are not involved. Whole-genome expression profiling in non-selective growth conditions showed that only a few genes were commonly up-regulated in all obtained silver-resistant mutants. In addition, deletion mutants and plasmid-based complementation confirmed that a two-component regulatory system and a gene coding for a small periplasmic protein play a central role. These results represent a previously uncharacterized molecular mechanism for metal/silver resistance.

FEMS7-1295
Physiology / Biochemistry / Molecular Microbiology

CLOSTRIDIUM DIFFICILE GENE ASSOCIATED WITH HAEMOLYTIC ACTIVITY IN ESCHERICHIA COLI CLONES

Z. Alkudmani[1], R. Williams[2], P. Mullany[1], A.P. Roberts[1]
[1]Eastman Dental Institute- University College of London, Microbial Diseases, London, United Kingdom
[2]University College of London, Infection and Immunity, London, United Kingdom

Backgrounds

It was observed that *Clostridium difficile* exhibit a haemolytic phenotype when grown on brain heart infusion (BHI) agar supplemented with 5% horse blood and 2% glucose. This phenotype however is not observed in the absence of glucose.

Objectives

To determine the genetic basis for haemolysin production in *C. difficile.*

Methods

A genomic library from *C. difficile* strain 630 was constructed and screened for haemolysis in *E. coli*. Multiple haemolytic colonies were identified and the most haemolytic was selected for further investigation. The gene responsible for the phenotype in that clone was localised. It was predicted to encode a protein containing an anthrax toxin lethal factor (ATLF) domain and a signal peptide.

Conclusions

E. coli clones carrying a *C. difficile* gene encoding an uncharacterized protein with an ATLF domain are haemolytic.

FEMS7-1334
Physiology / Biochemistry / Molecular Microbiology

COMPARATIVE CELL WALL ANALYSIS OF THE VIBRIONACEAE FAMILY

L. Alvarez[1], K. Kumar[1], I. Abreu[2], T. Moritz[2], F. Cava[1]
[1]MIMS- Umeå University, Molecular Biology Department, Umeå, Sweden
[2]Swedish University of Agricultural Sciences- Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Umeå, Sweden

Backgrounds

Most bacteria surround themselves with a protective cell wall to withstand cell turgor pressure and repel environmental insults. These tough cell walls are primarily made of a peptidoglycan (PG) exoskeleton called the murein sacculus. PG structure is not static, but continuously remodeled in response to environmental and physiological cues, which requires the coordinated actions of numerous PG-acting enzymes and regulatory proteins.

Objectives

Investigation of the cell wall biochemistry is imperative for understanding its biology and its role in growth, division, and morphogenesis. Here we explore the mechanisms responsible for this remodeling and its biological meaning.

Methods

For this purpose, we have developed a new method that allows ultrafast and sensitive PG analysis by in-line UPLC-MS/MS coupled to chemometric tools for identifying cell wall subjacent variability.

Conclusions

By these means, we have characterized the cell wall diversity within the Vibrionaceae family and analyzed how the cell wall chemistry fits in relation with established bacterial phylogeny and ecology. Our analysis has permitted the identification of several species-specific cell wall chemical features, which we are now characterizing in detail. The mechanistic and phenotypic study of these properties will provide new insights about the role of PG remodeling in the bacterial lifestyle and environment adaptability. Moreover, this will provide a unique opportunity to uncover new targets against infecting bacteria.

FEMS7-1829
Physiology / Biochemistry / Molecular Microbiology

PREVENTION OF TIGHT JUNCTION INJURY CAUSED BY EPEC IN INTESTINAL EPITHELIAL CELLS BY FACTORS RELEASED BY COMMENSAL AND PROBIOTIC ESCHERICHIA COLI STRAINS

C.S. Alvarez[1], M.J. Fabrega[1], M.A. Cañas[1], R. Gimenez[1], L. Baldoma[1], J. Badia[1]
[1]Facultat de Farmàcia i Ciències de l'Alimentació, Biochemistry and phisiology, Barcelona, Spain

Backgrounds

The gastrointestinal epithelial layer forms a physical and biochemical barrier that maintains segregation between host and microbiota and regulates intestinal permeability. The integrity of this barrier is in part supported by the establishment of tight junctions (TJs) between intestinal epithelial cells. Pathogens such as Enteropathogenic *Escherichia coli* (EPEC) disrupt this barrier. However, some gut microbes and probiotic bacteria modulate the barrier integrity by reinforcing TJs. *E. coli* Nissle 1917 (EcN) is a probiotic with a positive effect on the barrier integrity. We have previously shown that both outer membrane vesicles (OMVs) and soluble factors released by EcN and commensal ECOR63 reinforce the epithelial barrier by positive regulation of ZO-1 and claudin-14, and negative regulation of claudin- 2.

Objectives

Here we analyze the ability of OMVs and soluble factors released by EcN and ECOR63 to protect EPEC-induced TJs damage in T-84 and Caco-2 cell monolayers.

Methods

Transepithelial Electrical Resistance (TER) and distribution of TJ proteins were monitored in polarized cells infected with EPEC in the absence or presence of both extracellular fractions collected from EcN and ECOR63. EPEC caused 50% reduction in the TER levels and altered localization of ZO-1, ZO-2 and occludin. However, OMVs and soluble factors secreted by EcN or ECOR63 avoided TER reduction and promoted redistribution of the altered TJs proteins. The effect of these secreted factors was also evaluated measuring expression of miRNAs associated with the epithelial barrier function.

Conclusions

Results indicate the ability of OMVs and factors secreted by EcN and ECOR63 to protect TJs disruption caused by EPEC.

FEMS7-2216
Physiology / Biochemistry / Molecular Microbiology

COMPARISON OF SECOND INTERNAL TRANSCRIBED SPACER (ITS II) OF RIBOSOMAL DNA IN IDENTIFICATION AND DIFFERENTIATION OF TRICHOSTRONGYLUS SPECIES OF LIVESTOCK IN MAZANDARAN PROVINCE, IRAN

A. Amouei[1], A. Azadeh Mizani[2], A. Ali Bakooie Katrimi[3], S. Shirzad Gholami[4]
[1]Committee research student - Mazandaran University of Medical Sciences- Sari- Iran, Parasitology And Mycology, Sari, Iran
[2]Toxoplasmosis Research Center- Mazandaran University of Medical Sciences- Sari- Iran, Parasitology and Mycology, Sari, Iran
[3]Mazandaran Provincial Veterinary Department of Medical Sciences- Sari- Iran, Medical Sciences, Sari, Iran
[4]Parasitology and Mycology Department- Faculty of Medicine- Mazandaran University of Medical Scie nces- Sari- Iran, Medical Sciences, Sari, Iran

Backgrounds

Trichostrongylus is an intestinal parasite of wide range of animals and human with a worldwide distribution. Reliable differentiation of *Trichostrongylus* sp. is important for epidemiological researches and effective control strategies of the parasite.

Objectives

The aim of present study was to identify *Trichostrongylus* spp. among ruminants using molecular method, in Mazandaran Province, northern Iran.

Methods

180 adult T*richostrongylus* specimens of gastrointestinal track of sheep and goat were obtained from a slaughterhouse in Mazandaran Province. After extraction, DNA fragments of the rDNA ITS II region of *Trichostrongylus* adult worms were amplified by conventional PCR.

Based on nucleotide differences between T*richostrongylus* sp., for species differentiation, two enzymes *Hinf* I and *Dra* I were selected to perform PCR-RFLP assay.

Conclusions

Results indicated that 98 out of 180 samples were confirmed positive as *Trichostrongylus* genus with conventional PCR and *Trichostrongylus* specific primers successfully amplified a region of approximately 328bp in all Samples. After RFLP assay, the restriction enzyme *Dra* I produced two different patterns based on previous computer software results for *T. axeil T. colubriformis* (215, 110 bp) and *T. vitrines* (185, 145 bp). *Hinf* I has one cutting site for *T. colubriformis*, and has no cutting sites for two other species, producing two fragments approximately 238, 90 bp for *T. colubriformis*.

PCR-RFLP profile is simple, rapid and also reliable tool for differentiation all *Trichostrongylus* species, and can be used for diagnostic, clinical and epidemiological purposes. These method can also be adopted to identify human and animal samples in endemic region of disease.

FEMS7-2519
Physiology / Biochemistry / Molecular Microbiology

UNIQUE FEATURES IN THE CYANOBACTERIAL DIVISOME IN ANABAENA SP. PCC7120.

D.M. Andrade Molina[1], M. Silva[1], H. Osorio[1], J. Chichón[2], J.M. Valpuesta[2], M. Vicente[3], L.M. Vásquez[1]
[1]Pontificia Universidad Católica de Chile, Molecular genetic and Microbiology, Santiago, Chile
[2]Centro Nacional de Biotecnología- CSIC, Molecular Structure, Madrid, Spain
[3]Centro Nacional de Biotecnología- CSIC, Microbial biotechnology, Madrid, Spain

Backgrounds

Cyanobacteria are oxygenic photosynthetic microorganism with cell walls properties and cell division proteins combined of both gram-positive and gram-negative bacteria. The research in cyanobacterial division has been revealed that divisome presents differences in composition and regulation compared to classical division models of bacteria. Previous studies carried out in our laboratory, have determined a gene common to all filamentous cyanobacteria (*cyDiv*), which is involved in cell division.

Objectives

To determine how the CyDiv protein contributes to divisome ensemble in filamentous cyanobacteria *Anabaena* sp. PCC7120

Methods

We analyzed phenotypic variations under different transcription levels controlled by copper-inducible promoter and determined the localization protein by immunofluorescence and morphological effect by TEM and SEM. Furthermore, was evaluated association of CyDiv with other protein of divisome by BIFc and determined secondary structure of periplasmic region by circular dichroism.

Conclusions

Integral results from gene expression, cellular localization and protein interaction suggest that CyDiv is essential for cell division in cyanobacteria and perform its role through interaction with elements of divisome. Alteration of CyDiv in *Anabaena* PCC7120 led to effects in the Z-ring positioning and cell wall damage triggering cell lysis. We show that FtsQ hypothetical protein and CyDiv localized depending of time of division from pole to cell center. Subsequently, we determined that CyDiv interact directly with FtsQ, being functionally homologous to FtsB in *E. coli*. Biochemical characterization of CyDiv determined that the periplasmic region is represented by a-Helix, confirming that this protein has similar topology to FtsB. Our results suggest that CyDiv and FtsQ could be involved in the link between the early recruitment proteins and late proteins of Divisome at the periplasm in cyanobacteria. Furthermore, CyDiv is required to septal junction complex to apparently stabilize the filament, since the absence of this leads to fractionation of the filament, defects in cell division and cell death.

FEMS7-1521
Physiology / Biochemistry / Molecular Microbiology

PURIFICATION AND CHARACTERIZATION OF THE ENZYME 3-HYDROXY-3-METHYL GLUTARYL COENZYME A REDUCTASE FROM CANDIDA GLABRATA (REC-HMGRCG)

D. Andrade[1], R. Cuevas[1], J. Ortiz[1], J. Tamariz[2], C. Hernández[1], A. Ibarra[1], L. Vills[1]
[1]Instituto Politécnico Nacional, Microbiología, D.F., Mexico
[2]Instituto Politécnico Nacional, Química Orgánica, D.F., Mexico

Backgrounds

The enzyme 3-hydroxy-3- methyl glutaryl coenzyme A reductase (HMGR) is a glycoprotein involved in the route of biosynthesis of cholesterol in humans and of ergosterol in fungi. The catalytic domain of HMGRs is highly conserved in eukaryotic organisms. We used the HMGR enzyme of *Candida glabrata* (HMGRCg) as a model for studying different lipid-lowering compounds with potential use in humans, as well as for developing new antifungal agents against this opportunistic pathogen, which has innate resistance to azoles.

Objectives

To obtain the recombinant version of HMGRCg in the *E. coli* expression system and demonstrate its inhibition with synthetic drugs.

Methods

1. Primers were designed to amplify the sequence encoding the soluble fraction of HMGRCg. 2. The HMGRCg gene was amplified by PCR. 3. The HMGRCg gene was cloned in the expression vector pMAL-c2xa. 4. Rec-HMGRCg was overexpressed and purified. 5. Rec-HMGRCg was characterized.

Conclusions

We could clone, express and purify the HMGR protein from *C. glabrata* in the heterologous system of *E. coli*, and then to demonstrate its activity and inhibition with synthetic drugs. Rec-HMGRCg showed an optimum pH of 8.0 and optimum temperature of 37°C. The k_m and V_{max} for HMG-CoA were 6.5 µM and 2.26 x 10^{-3} µM min^{-1}, respectively. The recombinant enzyme HMGRCg was inhibited by simvastatin and synthetic compounds presenting a similar IC_{50}. These inhibition data were supported by docking studies. The results suggest that rec-HMGRCg can serve as a model for studying the antifungal activity of synthetic compounds.

FEMS7-0077
Physiology / Biochemistry / Molecular Microbiology

STRUCTURE AND FUNCTION OF THE HEME UPTAKE MACHINERY IN CORYNEBACTERIUM GLUTAMICUM

S. Aono[1], *N. Muraki*[1]
[1]*Institute for Molecular Science, Okazaki Institute for Integrative Bioscience, Okazaki, Japan*

Backgrounds

Heme uptake machinery of Corynebacteria including *Corynebacteria glutamicum* and *Corynebacterium diphtheriae* consists of heme binding proteins, HtaA and HtaB, and the ABC-type heme transporter HmuTUV. Sequence analysis identified a conserved region (CR) of approximately 150 amino acids that is duplicated in HtaA and present in a single copy in HtaB. HtaA consists of two homologous CRs in the N- and C-terminal regions.

Objectives

To elucidate the molecular mechanisms of heme uptake in Corynebacteria, we have determined the crystal structures of HtaA, HtaB, and HmuT.

Methods

Crystallization was carried out in a 96-well crystallization plate at 20 ºC by hanging drop vapor diffusion method. Diffraction data were obtained at 100 K on the beamline BL44XU at SPring-8.

Conclusions

We have determined the crystal structures of the N-, and C-terminal CR of HtaA (HtaA-N and HtaA-C, respectively) and HtaB at the resolution of 2.0, 1.3, and 1.7 Å, respectively. HtaA-N consists of 11 β strands and two short α helices and accommodates one heme molecule with Tyr58 located in the first α helix as the heme axial ligand. Tyr58 forms a hydrogen bond with His111.

HtaA-C and HtaB show similar global structures to HtaA-N. The key residues for heme-binding and recognition including the axial ligand of heme and residues involved in the hydrogen bonding interactions with heme are conserved among HtaA-N, HtaA-C, and HtaB.

We also determined the crystal structure of HmuT at the resolution of 1.4 Å. HmuT consists of structurally similar two domains located in the N-terminal and C-terminal regions connected a long a helix. A single heme molecule is bound in the cleft between these domains. Heme iron is ligated by His141 and Tyr240, and Tyr240 forms a hydrogen bond with Arg242. Intriguingly, HmuT binds a heme with two different orientations.

FEMS7-2385
Physiology / Biochemistry / Molecular Microbiology

HIGH GENOMIC AND METABOLIC DIVERSITY IN INFANT-DERIVED BIFIDOBACTERIUM LONGUM STRAINS

S. Arboleya[1], F. Bottacini[2], M. O'Connell-Motherway[2], C.A. Ryan[3], R.P. Ross[4], D. van Sinderen[2], C. Stanton[1]
[1]Teagasc Food Research Centre Moorepark, APC Microbiome Institute, Cork, Ireland
[2]APC Microbiome Institute, School of Microbiology, Cork CIty, Ireland
[3]APC Microbiome Institute, Department of Neonatology, Cork CIty, Ireland
[4]APC Microbiome Institute, School of Science- Engineering & Food Science, Cork CIty, Ireland

Backgrounds

Bifidobacterium longum is an abundant species in the human intestinal microbiota and is widely used as a probiotic. This species is highly competitive in the complex intestinal community and is commonly present at high numbers in the gut of infants, adults and the elderly, thus indicative of a close symbiotic host-microbe relationship with humans throughout life.

Objectives

The objective of this study was to assess the genetic and metabolic diversity in *B. longum* strains isolated from healthy infants.

Methods

Twenty infant-derived *B. longum* strains were subjected to whole genome sequencing and their phylogenetic relatedness and pan-genome was analysed *in silico*. To better understand this diversity, a comparative genomic analysis was performed using other publicly available *B. longum* genome sequences. The twenty strains were then assessed for their ability to utilize different carbohydrates in an attempt to distinguish strains based on their metabolic abilities with regards to a wide range of carbohydrates, from monosaccharides to complex sugars and human milk oligosaccharides (HMOs).

Conclusions

The results showed high diversity in terms of genes and predicted glycosyl-hydrolases, as well as the ability to metabolize a large range of sugars. Moreover, we corroborate the capability of *B. longum* spp. *longum* to metabolise HMOs. Ultimately, these observations may provide an explanation for the persistence of this species throughout life.

FEMS7-2475
Physiology / Biochemistry / Molecular Microbiology

COLE1 PLASMID COEXISTENCE IS MEDIATED BY COMPENSATORY ADAPTATION

M. Ares-Arroyo[1], C. Bernabé-Balas[1], A. Santos-López[1], A. San Millan[2], A. Hoefer[1], B. Gonzalez Zorn[1]
[1]Faculty of Veternary Medicine and VISAVET- Complutense Univesrity of Madrid, Department of Animal Health, Madrid, Spain
[2]Hospital Universitario Ramón y Cajal and Instituto de Investigación Sanitaria IRYCIS, Servicio de Microbiología, Madrid, Spain

Backgrounds

ColE1-like plasmids are small multicopy plasmids that are highly prevalent in nature. Despite the fitness cost they impose on their host, the coexistence of these replicons is a very common phenomenon. Furthermore, it has been described that this cohabitation can drive antimicrobial multiresistance. As such, understanding the behavior of these coexisting plasmids is of great significance.

Objectives

This study aims to characterize the selection for plasmid cohabitation without antibiotic pressure, despite the high biological cost associated, by evaluating two hypothesis: positive epistasis and compensatory adaptation.

Methods

As a model, we used *Haemophilus influenzae* RdKW20 and three different Cole1-like plasmids: pB1000, pB1005 and pB1006, bearing the genes bla_{ROB-1}, *strA* and *tet*(O), respectively. We performed experimental evolutions where the fitness cost of the replicons was determined by direct competition experiments. We also analyzed the plasmid copy number and the resistance level of the bacteria harboring the plasmid(s).

Conclusions

In our study, pB1000, pB1005 and pB1006 maintained their resistance phenotype, plasmid copy number and fitness cost, whether alone or in combination. Thus, the positive epistasis hypothesis cannot explain the cohabitation phenomenon, as there is no fitness advantage associated with plasmid coexistence. However, by performing experimental evolutions, *H. influenzae* Rd was able to completely compensate for the fitness cost associated with one of these ColE1 plasmids. Once the bacterium had compensated this first replicon, the acquisition of the other ColE1 plasmid(s) did not produce any additional fitness cost. Therefore, compensatory adaptations explains the acquisition and coexistence of multicopy plasmids in nature.

FEMS7-1232
Physiology / Biochemistry / Molecular Microbiology

MRKH ANTAGONIZES H-NS-DEPENDENT REPRESSION IN KLEBSIELLA PNEUMONIAE MRKJ

M.A. Ares[1], J.L. Fernández-Vázquez[2], S. Pacheco[3], V.I. Martínez-Santos[4], M.D. Jarillo-Quijada[2], J. Torres[1], M.D. Alcantar-Curiel[2], J.A. González-y-Merchand[5], M.A. De la Cruz[1]
[1]Instituto Mexicano del Seguro Social, Unidad de Investigación en Enfermedades Infecciosas, Mexico city, Mexico
[2]Universidad Nacional Autónoma de México, Unidad de Medicina Experimental, Mexico city, Mexico
[3]Universidad Nacional Autónoma de México, Departamento de Microbiología Molecular, Cuernavaca, Mexico
[4]Universidad Autónoma de Guerrero, Unidad Académica de Ciencias Quimico Biológicas, Chilpancingo, Mexico
[5]Instituto Politécnico Nacional, Departamento de Microbiología, Mexico city, Mexico

Backgrounds

Klebsiella pneumoniae is a common opportunistic pathogen causing nosocomial infections. One of the main virulence determinants of *K. pneumoniae* is the type 3 pilus (T3P). T3P is genetically organized in three transcriptional units: the *mrkABCDF* polycistronic operon, the *mrkHI* bicistronic operon and the *mrkJ* gene. MrkH is a regulatory protein encoded in the *mrkHI* operon, which positively regulates the *mrkA* pilin gene and its own expression. In contrast, the H-NS nucleoid protein represses the transcriptional expression of T3P. No reports have been described for the role of MrkH protein in regulating *mrkJ* promoter in *K. pneumoniae.*

Objectives

Determine the role of MrkH and H-NS in transcription of *mrkJ* promoter.

Methods

Transcriptional expression of *mrkI* gene was carried out by qRT-PCR. Using EMSA, promoter region (PCR) of *mrkJ* was incubated with purified MrkH protein and DNA-proteins complexes were observed.

Conclusions

MrkH and H-NS positive and negatively regulated *mrkJ* expression, respectively, by binding to the promoter of *mrkJ*. MrkH acted as an anti-repressor of H-NS. Moreover, our results support that high levels of MrkH represses *mrkJ* expression. Our data provide new insights about the complex regulatory role of the MrkH protein on the transcriptional control of T3P in *K. pneumoniae*.

FEMS7-2059
Physiology / Biochemistry / Molecular Microbiology

INTESTINAL MICROBIOTA DYSBIOSIS AND EMERGING INFLAMMATION CAUSED BY A HIGH FAT DIET

A. Nerea[1], P. Miguel Angel[1], A. Laura[2], R. Andoni[3], R. Alfonso[4], G. Ana[5]
[1]Azti tecnalia, Food research, Derio, Spain
[2]Azti tecnalia, marine research, Sukarrieta, Spain
[3]EHU/UPV, Immunology- microbiology and parasitology, Leioa, Spain
[4]Instituto hispalense de pediatría, pediatría, Sevilla, Spain
[5]Instituto hispalense de pediatría, Gastroenterología, Sevilla, Spain

Backgrounds

A number of functions required for host survival are encoded by human core microbiome, being among others digestion, restriction of the growth of potentially harmful bacteria, or development of the immune response. The role that microbes have in energy homeostasis has driven to study their association with diseases such as diabetes, obesity or Alzheimer.

As we learn more about microbiome composition and functions in healthy individuals, it will become possible to use the microbiome as a novel target for diagnostic and therapeutic applications. This microbial diversity can be modified by genetic and environmental factors, including dietary habits and physical activity that have been shown to play a decisive role on defining the intestinal microbiota profile.

Recent studies have focused on the microbial environmental factor that pre-disposes humans to obesi. The obesity epidemic is characterized by an excessive accumulation of body fat and a low-grade systemic inflammatory tone. Energy-dense foods and overnutrition represent major starting points altering the lipid metabolism, systemic inflammation and gut microbiota.

Objectives

To elucidate the relationship between the gut microbiota dysbiosis and the emerging metainflammation caused by high fat diet consumption.

Methods

Adult zebrafish individuals were maintained during 6 weeks with either a control diet or a high fat diet. Intestinal microbiota dysbiosis was studied by 16S sequencing and the total number of bacteria by qPCR. Furthermore, intestinal inflammation was studied by gene expression profile and histology.

Conclusions

A HFD causes changes in the intestinal microbiota composition closely related to an emerging inflammation in adult zebrafish.

FEMS7-2714
Physiology / Biochemistry / Molecular Microbiology

ZEBRAFISH GUT AS A HOUSE FOR HUMAN INTESTINAL BACTERIA

M.A. pardo[1], N. Arias[1], A. Ramirez[2], A.P. Galera[3], A. Rodriguez[4], L. Alonso[5]
[1]azti tecnalia, food research division, Derio, Spain
[2]EHU/UPV, immunology- microbiology and parasitology, Leioa, Spain
[3]IHP, AE gastroenterología, Sevilla, Spain
[4]IHP, Pediatría, Sevilla, Spain
[5]Azti tecnalia, Marine research divisin, Sukarrieta, Spain

Backgrounds

The human gut houses a vast microbial community that is vital for maintaining host health. The complexity and the high inter-individual variability of the human gut microbiota are inherent problems in the study of host-microbe interactions.

Gnotobiotic animals offer the opportunity to circumvent some problems. For that reason, it would be desirable to have a simple animal model to study the interactions between the gut microbiota and the host. Zebrafish is a vertebrate model that is being widely used to investigate the interactions between a host and its resident microbial communities because it is genetically similar to humans and easier and cheaper to house and care for than rodents.

Objectives

To know which species directly obtained from the human gut microbiota are able to colonize the zebrafish gut.

Methods

We have colonized the intestine of 5 days post fertilization (dpf) zebrafish larvae with human intestinal microbiota. A thorough protocol is followed to obtain axenic zebrafish larvae that are infected with the microbiota during 48 hours. The colonization is monitorized by 16S sequencing.

Conclusions

Certain interenting strains included in the human intestinal microbiota are able to colonize the zebrafish gut almost during 48 hours post infection (hpi). As a matter of fact, we resolved that different bacteria commonly found in the human intestine, including obligate anaerobes, are able to colonize and compete into the zebrafish intestine.

These data suggest that the developing zebrafish could be a suitable model for studying few species of the human gut microbiota, the interactions with the host, and the related diseases, as a result, diabetes, obesity, IBD.

FEMS7-1858
Physiology / Biochemistry / Molecular Microbiology

TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF RIBOSOMAL PROTEIN OPERONS IN ESCHERICHIA COLI

L. Aseev[1], L. Koledinskaya[1], I. Boni[1]
[1]M.M. Shemyakin and Yu.A. Ovchinnikov Institute of bioorganic chemistry, Department of Genetics and Postgenomic Technologies, Moscow, Russia

Backgrounds

Ribosome biogenesis in bacteria requires the coordinated synthesis of three rRNAs (16S, 23S and 5S) and more than 50 ribosomal proteins. While regulation of rRNA and about a half of r-protein operons in *E. coli* has been already well defined, a number of r-protein operons remain largely unexplored. It is important to obtain a deeper understanding of the regulatory mechanisms responsible for balanced synthesis of all ribosomal components.

Objectives

Our main goal was to characterize the mechanisms regulating expression of three previously unexplored r-protein operons, *rplM-rpsI, rplU-rpmA*, and *rpmB-rpmG* which encode r-proteins L13-S9, L21-L27, and L28-L33, respectively.

Methods

To study transcriptional control, we evaluated the changes in amounts of the transcripts in total RNA isolated before and after induction of serine starvation by treatment with L-serine hydroxamate of wild-type cells as well as ppGpp-null and *dksA* mutants. For this purpose, RT-qPCR with an external RNA standard was used. Translation regulation has been examined by measuring expression of the operon-specific chromosomal *lacZ* fusions under normal versus augmented synthesis of operon products.

Conclusions

Under amino acid starvation, transcription of all three operons is subject to ppGpp/DksA–dependent negative stringent control, in parallel with the rRNA operons. At the translational level, only one of these operons, *rplM-rpsI*, is regulated by the mechanism of autogenous repression, indicating that translational feedback control is not a general rule for modulating r-protein expression. L13, a primary protein in 50S subunit assembly, serves as an autogenous repressor of the *rplM-rpsI* mRNA by recognizing the complex structure of the *rplM* 5'-UTR.

FEMS7-1090
Physiology / Biochemistry / Molecular Microbiology

DOMAIN OF UNKNOWN FUNCTION IS INVOLVED IN THE CONVERSION OF 2-THIOURACIL INTO URACIL

A. Aučynaitė[1], R. Rutkienė[1], R. Meškys[1], J. Urbonavičius[1,2]
[1]Vilnius university Institute of Biochemistry, Department of Molecular Microbiology and Biotechnology, Vilnius, Lithuania
[2]Vilnius Gediminas Technical University, Department of Chemistry and Bioengineering, Vilnius, Lithuania

Backgrounds

Modified nucleotides are present in many RNA species in all Domains of Life. The biosynthetic pathways of such nucleotides are well studied. However, much less is known about the degradation of RNAs and the salvage of modified nucleotides, their respective nucleosides or heterocyclic bases to the metabolism.

Objectives

To screen the metagenomic libraries for genes, which would allow the conversion of 2-thiouracil to uracil in *E. coli* cells.

Methods

Using an *E. coli* uracil auxotrophic strain, we screened the metagenomic libraries for genes, which would allow the conversion of 2-thiouracil to uracil and thereby lead to the growth on a defined synthetic medium. We show that a novel gene encoding previously uncharacterized Domain of Unknown Function (DUF) is responsible for such phenotype. We have purified this recombinant protein and demonstrated that it contains a Fe-S cluster. The substitution of cysteines, which have been predicted to bind such clusters, with alanines abolished the growth phenotype.

Conclusions

We conclude that previously uncharacterized Domain of Unknown Function is required for conversion of 2-thiouracil into uracil *in vivo*.

FEMS7-1092
Physiology / Biochemistry / Molecular Microbiology

ROEMERINE: A NEW MULTIDRUG RESISTANCE PUMP INHIBITOR?

F.G. Avci[1], M. Guillerminet[2], C. Orelle[2], J.M. Jault[2], B. Sariyar-Akbulut[1]
[1]Marmara University - Faculty of Engineering, Department of Bioengineering, Istanbul, Turkey
[2]University Lyon- CNRS, UMR5086-Molecular Microbiology and Structural Biochemistry, Lyon, France

Backgrounds

Increasing resistance against current antimicrobials leads to loss in drug efficacy.Bacteria reduce the effects of drugs using various resistance mechanisms that include efflux through multidrug resistance (MDR) pumps. Combinatorial therapies of antimicrobials with pump inhibitors are a promising strategy to recover sensitivity to current antimicrobials.

Objectives

(-)-Roemerine is an aporphine type alkaloid with significant antibacterial and antifungal activities. It has been reported to increase the cytotoxicity of vinblastine in multidrug-resistant KB-V1 cells by interacting with the eukaryotic MDR pump, P-glycoprotein. In the light of this information our study investigates the possible modulation of the activity of P-glycoprotein homologue BmrA in *Bacillus subtilis* 168 by aporphine type alkaloids, (-)-roemerine, boldine, and bulbocapnine.

Methods

Growth curves were obtained for *Bacillus subtilis* 168 cells in the presence of berberine and the inhibitors. Berberine was used as the model antimicrobial agent and its concentration was kept at 75 µg/mL. The inhibitor concentrations were kept at 25 µg/mL. Furthermore inverted membrane vesicles were constructed with wild-type BmrA to test inhibition of the ABC transporter of *B. subtilis* 168.

Conclusions

75 µg/mL berberine had no significant effect on microbial growth of *B. subtilis* 168 cells. The combination of 75 µg/mL berberine with 25 µg/mL (-)-roemerine retarded cell growth. No effect was observed in berberine combinations with boldine or bulbocapnine. When the effects of berberine, boldine, and (-)-roemerine were tested for their inhibition of wild-type BmrA overexpressed in *E. coli* inverted membrane vesicles, the results showed that (-)-roemerine selectively bound and blocked the function of BmrA.

FEMS7-0473
Physiology / Biochemistry / Molecular Microbiology

STRUCTURAL AND BIOPHYSICAL CHARACTERIZATION OF A NOVEL LYSOZYME INHIBITOR FROM NEISSERIA MENINGITIDIS

A. Awanye[1], V. Humbert[2], L.Y. Lian[3], M. Christodoulides[2], J. Derrick[1]
[1]University of Manchester, Faculty of Biology- Medicine and Health, Manchester, United Kingdom
[2]University of Southampton, Faculty of Medicine, Southampton, United Kingdom
[3]University of Liverpool, Institute of Integrative Biology, Liverpool, United Kingdom

Backgrounds

Neisseria meningitidis is a Gram negative bacterium which can cause septicaemia and meningitis. One mechanism which may promote its survival in the nasopharynx is inhibition of endogenous lysozyme activity. The Adhesin Complex Protein (ACP) is a surface exposed and highly conserved outer membrane protein with the ability to confer protection against meningococcal infections (Hung et al, mBio 4(2):e00041-13).

Objectives

We report the structural and biophysical characterization of ACP and demonstrate that it is a potent inhibitor of human lysozyme.

Methods

Recombinant ACP was expressed in *E. coli* and purified by Ni-IDA affinity, cation exchange and size exclusion chromatography (SEC). Binding between ACP and lysozyme was demonstrated by SEC and verified using microscale thermophoresis; the equilibrium binding constant (K_D) was 11 μM. The lysozyme inhibitory activity of ACP was assessed using a fluorimetric method; it was observed that ACP is a stronger inhibitor of human lysozyme (92% inhibition), compared to hen egg white lysozyme (82% inhibition). Crystals of ACP were obtained by the sitting drop method and native data collected to 1.35Å resolution. The crystal structure was solved using anomalous diffraction phasing from iodide ions, and gave a single molecule in the asymmetric unit. The structure of ACP revealed an 8-stranded β–barrel structure, with several loop regions.

Conclusions

ACP is structurally similar to *Pseudomonas* MliC, *Brucella* and *Salmonella* PliC although it does not share significant primary sequence similarity (less than 25%) or described sequence motifs with either protein. Our results suggest that ACP is the first lysozyme inhibitor to be identified from *Neisseria*.

FEMS7-1998
Physiology / Biochemistry / Molecular Microbiology

COMPARATIVE MIRNAOME ANALYSIS IN HIV-1 INFECTED PATIENTS REVEALED ALTERED MIRNA EXPRESSION PROFILES IN ELITE CONTROLLERS AND LONG-TERM NON PROGRESSORS

R. Ayala Suárez[1], E. Calonge[1], H.E. De la Torre Tarazona[1], F. Díez Fuertes[1], J. Alcami Pertejo[1]
[1]Centro Nacional de Microbiología - Instituto de Salud Carlos III, AIDS Immunopathology Unit, Majadahonda- Madrid, Spain

Backgrounds

Long-term non progressors (LTNP) and elite controllers (EC) represent spontaneous models of efficient immune response against HIV-1 due to their capacity to maintain CD4>500 cell/µL for more than 10 years and to control viral replication in the absence of treatment, respectively. Altered expression of some miRNAs has previously been associated with these phenotypes.

Objectives

The main purpose of this work is to characterize the miRNAome of HIV-1 infected patients with extreme phenotypes (EC-LTNP and LTNP) and to identify different miRNA expression profiles.

Methods

Thirty miRNAomes of PBMCs collected from EC-LTNP (n=8), viremic LTNP (n=8) and typical progressors (n=7) before (TP) and after receiving antiretroviral therapy (TP-ART) were sequenced by miRNA-Seq and subsequently analysed to find differential expression profiles. A mean of 13.211.670 reads per patient were aligned to the miRNAome using miRanalyzer, obtaining an average of 472 pre-miRNAs and 355 miRNAs. Differential expression analyses between phenotypes were conducted by DESeq2.

Conclusions

A total of 15 miRNAs were deregulated comparing LTNP with TP, including the downregulation of miR-144/451 cluster in LTNP, which has previously been associated with the inhibition of CCR5 agonists, and the downregulation of miR-18a in LTNP, which regulates Dicer expression and the canonical-maturation of miRNAs. A deregulation of 43 miRNAs was observed when EC-LTNP and viremic-LTNP patients were compared, including the downregulation of miR-29a in EC-LTNP, associated with the inhibition of Nef protein. The downregulation of miR-146a/miR-106 and the upregulation of miR-30a in EC-LTNP were also detected and linked to an enhanced immune response in these individuals.

FEMS7-1708
Physiology / Biochemistry / Molecular Microbiology

EXPERIMENTAL AND BIOINFORMATIC ANALYSES OF STRESS TOLERANT AND RESPONSE ON ACETIC ACID BACTERIA

Y. Azuma[1]
[1]*Kindai University, Biology-Oriented Science and Technology, Kinokawa, Japan*

Backgrounds

Acetic acid bacteria (AAB) are obligate aerobic and Gram-negative bacteria, and taxonomically belong to the family *Acetobacteraceae* of alpha-proteobacteria. *Acetobacter pasteurianus* and *Gluconacetobacter* species of AAB are historically and world-widely used for vinegar fermentation, and many kinds of AAB were used in a variety process of food fermentation, including precursors of vitamin C, chocolate, and kombucha.

Objectives

Despite the industrial values, AAB show noticeable physiological instability or high adaptation potential under stress environments in two means, i.e. temporal acclimation and heritable adaptation. To clarify genomic backgrounds and take advantage of the genetic flexibility, comparative omics analyses were performed.

Methods

For temporal acclimation and heritable adaptation analyses, comparative omics analyses were performed using hyper glucose-tolerant AAB, *Tanticharoenia sakaeratensis* and *Asaia bogorensis*, and using an adapted strain of *A. pasteurianus* at unviable high-temperature, respectively.

Conclusions

The two hyper glucose-tolerant AAB, *T. sakaeratensis* and *A. bogorensis* were shown to alter expressions of homologous genes and systems under higher glucose-concentration conditions, especially repression of glycolysis and pentose phosphate pathways and induction of antioxidant enzymes, such as SOD and peroxidases. Interestingly the involvement of antioxidant enzymes in the thermo-tolerant adaptation was observed, and some of the genes were common with ones involved in high glucose-tolerance. Based on the fact that enzymes managing oxidative stresses were also engaged in overcoming different stresses, such as the high glucose stress and thermo-stress, we propose a hypothesis that disruption of cell homeostasis by oxidative stress is a general reason for death or quiescence under a variety of stress conditions.

FEMS7-1387
Physiology / Biochemistry / Molecular Microbiology

QNRD IS SOS REGULATED BY SUB-MIC TOBRAMYCIN IN ESCHERICHIA COLI

A. Babosan[1], L. François[2], D.C. Christophe[1], P. Gerald B.[3], S. David[3], G. Thomas[1]
[1]University of Reims Champagen Ardenne, UFR Medecine, Reims, France
[2]Massachussets Eye and Ear Infirmary- Harvard Medical School, Microbiology and Immunology, Boston, USA
[3]Channing Laboratory- Brigham and Women's Hospital- Harvard Medical School, Infectious Diseases, Boston, USA

Backgrounds

Little is known about the regulation of *qnrD*, which confers low-level resistance to fluoroquinolones (FQ), and carried, by a small plasmid in *Proteeae.*

Objectives

We investigated SOS transcriptional regulation of *qnrD,* after treatment with sub-inhibitory concentrations of ciprofloxacin (CIP), mitomycin C (MMC) and tobramycin (TM).

Methods

The expression of *qnrD* transcripts was measured using qRT-PCR, in *E. coli* MG1656 (WT) and derivatives, after exposure to CIP, MMC and TM. The fold change in *qnrD* gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. We performed growth with or without antibiotics, allowing reactive oxygen species (ROS) or nitric oxide (NO) detection.

Conclusions

In the WT strain, *qnrD* transcript expression was increased with SOS inducers CIP and MMC. In two strains were the SOS system was blocked, no transcript expression was found. These results indicated that *qnrD* expression is mediated by the SOS response.

Strikingly, *qnrD* transcript expression in the WT was induced by TM, whereas no response was observed in SOS blocked strains, showing that TM induces also the SOS response in *E. coli*.

No ROS was measured for *qnrD* plasmid-carrying bacteria with CIP or TM, whereas NO was detected when TM was added. In *qnrD* plasmid-carrying strains, TM induced a nitrosative stress that damage the DNA, thus SOS response and repair.

Overall, our findings revealed an unexpected antibiotic resistance co-selection with AG that may promote emergence of FQ resistance. This could be a worst-case scenario, enhancing the risk for emergence of high-level resistance to FQ in Qnr-producing *E. coli*.

FEMS7-1221
Physiology / Biochemistry / Molecular Microbiology

DNA REPAIR PATHWAYS INVOLVED IN THE RESPONSE TO LOW DOSES OF AMINOGLYCOSIDES IN E. COLI AND V. CHOLERAE

Z. Baharoglu[1]
[1]*Institut Pasteur, Genomes et genetique, paris, France*

Backgrounds

Antibiotic treatments challenge bacteria to develop new survival strategies. Antibiotics at low doses that do not affect bacterial growth were found to induce various stress responses in Gram-negative bacteria. Particularly, low concentrations of aminoglycosides induce the SOS response in *Vibrio cholerae* but not in *Escherichia coli*.

Objectives

Our goal is to study the strategies adopted to respond to such antibiotic stress, through the characterization of molecular responses involved, and the study of subsequent DNA damages.

Methods

In order to determine whether a specific factor present in *E. coli* prevents this induction, we developed a genetic screen where only SOS inducing mutants are viable. We identified the vsr gene coding for the Vsr protein of the very short patch mismatch repair (VSPR) pathway. The effect of mismatch repair (MMR) mutants was also studied. To further understand the mechanisms involved in the response to aminoglycosides in *V. cholerae*, we have also constructed large mutant libraries in order to identify genes that become essential in the presence of such stress.

Conclusions

We propose that lesions formed upon aminoglycoside treatment are preferentially repaired by VSPR without SOS induction in *E. coli* and by MMR when VSPR is impaired. The identification of the molecular mechanisms triggered by the presence of sub-MIC antibiotics will point to physiological processes that are important for the induction of stress responses and bacterial adaptation.

FEMS7-1852
Physiology / Biochemistry / Molecular Microbiology

A NEW CLASS OF ICE IN THERMUS THERMOPHILUS

I. Baquedano[1], *A. Blesa*[1], *M. Mencía*[1], *J. Berenguer*[1]
[1]*Centro de Biología Molecular Severo Ochoa UAM-CSIC, Molecular biology, MADRID, Spain*

Backgrounds

Integrative and Conjugative Elements (ICEs) are found in many bacteria. They frequently encode genes required for their insertion and excision and also for their mobilization from an *ori*T sequence and conjugal transfer (Type 4 secretion, T4SS) to a new host. In contrast with well known conjugation models, in some Thermus thermophilus strains a very active cell-to-cell DNA transfer mechanism exists that depends on the combination of a DNA secretion system (Push system) in the donor and a DNA incorporation system (pull system) in the recipient, both playing and active role in a process named transjugation. The pull system is the same used for natural competence, whereas the push system requires a DNA translocase of the FtsK family (CptA) encoded within a region of significantly lower G+C content than the genome.

Objectives

To analyse if the DNA-push system in transjugation is encoded by a mobilizable element integrated into the genome of *Thermus thermophilus*.

Methods

DNA transfer assays with mutants in different genes around the DNA pushing translocase. Integration and excision assays detected by specific PCR and qPCR.

Conclusions

The CptA protein is encoded by a new type of ICE that integrates at and excises from a specific tRNA in the genome. In addition to its own transfer, this new class of ICE promotes the massive transfer of genes from its host to a recipient competent cell by transjugation.

This work has been funded by Spanish national project BIO2016-77031-R. IB is the holder of a fellowship from the MINECO

FEMS7-2447
Physiology / Biochemistry / Molecular Microbiology

BREAKING THROUGH THE STRESS BARRIER: THE ROLE OF BOLA IN FLAGELLAR SYNTHESIS AND BIOFILM FORMATION

S. Barahona[1]
[1]*Instituto de Tecnologia Química e Biológica António Xavier, MostMicro, Oeiras, Portugal*

Backgrounds

Bacteria are extremely versatile organisms, which rapidly adjust their gene expression to induce physiological and molecular adaptation to changing environments. When bacterial cells switch from planktonic to biofilm state of growth, flagella formation is turned off, and the production of fimbriae and extracellular polysaccharides is activated. BolA protein is widespread in nature and has been associated with several cellular processes.

Objectives

Unraveling the overall picture of BolA global effects and specific targets.

Methods

High-troughput techniques, such as Microarrays and ChIP-seq, have showed that BolA protein is a new bacterial transcription factor, which regulates the switch between motile and sessile lifestyle.

Conclusions

BolA negatively modulates flagellar biosynthesis and swimming capacity in *Escherichia coli*. Moreover, its overexpression favors biofilm development, involving fimbriae-like adhesins and curli production. Our recent results show that BolA action in these pathways is related with c-di-GMP a relevant intracellular signaling molecule involved in biofilm formation. We demonstrate that BolA contributes to a fine-tuned expression of different diguanylate cyclases and phosphodiesterases and c-di-GMP has a negative influence in the *bolA* mRNA transcription. Herein we propose that BolA is a key player in motile/adhesive transcriptional switch, contributing to a fine-tuned regulation of these important pathways.

FEMS7-1088
Physiology / Biochemistry / Molecular Microbiology

INTEGRATIVE CONJUGATIVE ELEMENTS IN MINIMAL BACTERIA: FROM INDIVIDUAL TO COLLECTIVE BEHAVIOR

E. Baranowski[1], E. Dordet-Frisoni[1], E. Sagne[1], M.C. Hygonenq[1], G. Pretre[1], S. Claverol[2], L. Fernandez[1], X. Nouvel[1], C. Citti[1]
[1]INRA/ENVT, UMR1225 IHAP, Toulouse, France
[2]Université de Bordeaux, Pôle Protéomique, Bordeaux, France

Backgrounds

The evolution of the wall-less mycoplasmas was long thought to be only driven by genome reduction, resulting in current mycoplasmas having a minimal genome. Evidences of massive gene flows within and across mycoplasma species provided a new frame to understand the successful adaptation of these minimal bacteria to a broad range of environments. This phenomenon was discovered in *Mycoplasma agalactiae* that contains an Integrative Conjugative Element (ICEA) belonging to a new clade of the Mutator-like superfamily in prokaryotes. Unlike classical ICEs, ICEA has no preferential insertion specificity and multiple copies can be found at different loci of the host chromosome.

Objectives

The overall objective of this study was to address ICEA functions and interactions among co-resident ICEAs to propose a model of conjugative transfer in mycoplasmas.

Methods

This was done by combining classical matting experiments to functional genomics.

Conclusions

The individual behavior of co-resident ICEAs was supported by complementation studies showing that conjugative-deficient ICEAs can only be plasmid-complemented despite the co-occurrence of multiple chromosomal ICEA copies. One exception was CDS14 knock-out ICEAs whose conjugative transfer was conditioned by the occurrence of additional ICEA copies. CDS14 encodes a lipoprotein expressed at the surface of ICEA positive cells and may participate in the early steps of the conjugative process. Remarkably, CDS14 expression in the ICEA-negative mating partner was able to restore the conjugative phenotype of an ICEA-positive partner carrying a single CDS14 knock-out ICEA. The complex behavior of co-resident ICEAs raises questions regarding the dynamics and evolution of these conjugative elements, including vestigial ICEAs.

FEMS7-0265
Physiology / Biochemistry / Molecular Microbiology

GENETIC DISSECTION OF PROTEIN SYNTHESIS INITIATION IN MTB REVEALS WHICH GENES ARE ESSENTIAL, SUGGESTING THE UNIVERSAL ESSENTIALITY OF METHIONINE AMINOPEPTIDASE LEYS IN AN UNIDENTIFIED ACTIVITY

D. Barkan[1], *M. Vanunu*[1]
[1]*The Hebrew University of Jerusalem - The Faculty of Agriculture, veterinary medicine, Rehovot, Israel*

Backgrounds

Protein synthesis initiation differs in prokaryotes and eukaryotes. In prokaryotes, the initiating methionine is formylated (by Formyl-methionine transferase, *fmt*), then de-formylated (by Peptide deformylase, *def*). Then, this methionine is removed by methionine peptidases (MAP) – a process essential in al life forms. In Mtb, two genes encode for MAP (*mapA, mapB*), with conflicting evidence as to which of them is essential.

Objectives

To systemically dissect the genetics of this metabolic pathway by targeted deletion of *fmt*, *def, mapA* and *mapB* in *M. smegmatis* and *Mtb*

Methods

For *fmt*, we created merodiploid mutanst in *M. smegamis* and Mtb, performed targeted deletions, then removed the complementing copy – by silencing and cassette-switch. For *mapA* and *mapB*, we created merodiploid strains, deleted the native gene, then exchanged the complementing for either an empty vector, a functional, or several mutated versions. We created double deletion mutants of both genes

Conclusions

fmt was completely dispensable in *M. smegmatis*, but essential in Mtb. *def* is essential in *M. smegmatis*. We unequivocally show that *mapB*, and not *mapA* is the essential one. **Despite complete essentiality, *mapB* could be replaced by at least 2 mutated, enzymatically inactive, versions**.

fmt essentiality differs in M.smegmatis and Mtb. *def* is essential in *M. smegmatis*, but revertant rate limits usefulness as drug target. *mapB*, and not *mapA*, is the essential MAP in Mtb. Most important, we suggest **that in Mtb – and perhaps in all life forms – MAP essentiality may not ley in their characterized activity, but in some other unidentified property or activity**.

FEMS7-1325
Physiology / Biochemistry / Molecular Microbiology

INVESTIGATING THE RELATIONSHIP BETWEEN QUORUM SENSING, THE TYPE III SECRETION NEEDLE-TIP PROTEIN LCRV AND AUTO-AGGREGATION IN YERSINIA PSEUDOTUBERCULOSIS

N.A. Barratt[1], A. Wiechmann[2], J. Purves[3], P. Williams[1], S. Atkinson[1]
[1]University of Nottingham, Centre for Biomolecular Sciences, Nottingham, United Kingdom
[2]Current Address Goethe University, Department of Molecular Microbiology and Bioenergetics, Frankfurt am Main, Germany
[3]Current Address University of Leicester, Department of Genetics, Leicester, United Kingdom

Backgrounds

The enteropathogen Y. pseudotuberculosis possesses a 70 kb virulence plasmid (pYV) that encodes a Type 3 Secretion (T3S) system, consisting of a needle-like injectisome that delivers effector proteins into host cells triggering apoptosis.

Y. pseudotuberculosis uses a quorum sensing (QS) system to control gene expression, which requires the density dependent production and transduction of N-acylhomoserine lactone (AHL) signal molecules, which accumulate with increasing cell number. AHLs are synthesised by the LuxI family of synthase proteins and transduced by LuxR response regulators. Y. pseudotuberculosis has two LuxR/I homologues, YtbR/I and YpsR/I, which regulate virulence related phenotypes, including the regulation of components of the T3S system, biofilm production and cellular auto-aggregation (A-Ag).

The T3S system, pYV and QS have been linked to YadA independent A-Ag since an AHL negative, but pYV positive, ytbI/ypsI double mutant shows a considerable increase in A-Ag compared to the parent. However, when the T3S needle protein YscF is mutated or when the double mutant is cured of pYV A-Ag is abolished.**Objectives**

To extend our knowledge of the relationship between QS, the T3S system and A-Ag the T3S needle-tip protein LcrV was mutated and A-Ag assessed in an AHL positive or negative background.

Methods

lcrV single and ytbI/ypsI/lcrV triple mutants were constructed using the suicide plasmid pDM4 and the ability of the newly constructed mutants to A-Ag was assessed using a Coulter-LS230 laser diffraction particle size analyser.

Conclusions

The lcrV mutant was unable to aggregate, whereas ytbI/ypsI/lcrV mutant showed restoration of the aggregation phenotype, suggesting that QS regulates A-Ag in a LcrV dependent manner.

FEMS7-2446
Physiology / Biochemistry / Molecular Microbiology

DECIPHERING THE ROLE OF A NEW STREPTOCOCCUS PNEUMONIAE PLAYER IN RIBOSOME ASSEMBLY AND TRANSLATION

C. Bárria[1]
[1]*Instituto de Tecnologia Química e Biológica António Xavier, MostMicro, Oeiras, Portugal*

Backgrounds

Ribosomes are macromolecular machines that translate mRNA into functional proteins. In bacteria, the 70S ribosome is composed of two subunits, a small 30S subunit and a large 50S subunit. Each subunit is composed of ribosomal RNA and ribosomal proteins. A proper and coordinated assembly of these players is crucial to form an active ribosomal particle.Ribonucleases (RNases) are enzymes that ensure maturation, degradation and quality control of RNA.

Objectives

Escherichia coli RNB family of enzymes is present in all domains of life and includes RNase R, RNase II and the eukaryotic Rrp44/Dis3, Dis3L1 and Dis3L2 proteins. In *Streptococcus pneumoniae* only RNase R was identified. RNase R level is increased in several stress conditions such as heat shock, stationary phase or cold shock, conditions in which most of the proteins translation is blocked.

Here, we investigated the role of RNase R in translation by comparing the wild type strain with an *rnr* mutant strain.**Methods**

We compared the ribosomal profile between the two strains using sucrose gradient separation and Western blots. We have also performed Northern blots analysis of transcripts involved in translation and ribosome assembly.

Conclusions

In this study we show that RNase R interacts with ribosomes mostly with the 50S subunit. Moreover, in the absence of this enzyme we have observed a decrease in the amount of the 70S ribosomal subunit, concomitantly with the increase of 50S subunit. RNase R seems also to be involved in the regulation of other RNases responsible for the processing of ribosomal RNA.

FEMS7-3114
Physiology / Biochemistry / Molecular Microbiology

GENOME-WIDE ANALYSIS OF PARB BINDING SITES IN PSEUDOMONAS AERUGINOSA

A.A. Bartosik[1], *K. Glabski*[1], *A. Kawalek*[1], *G. Jagura-Burdzy*[1]
[1]*Institute of Biochemistry and Biophysics- Polish Academy of Sciences, Department of Microbial Biochemistry, Warsaw, Poland*

Backgrounds

ParA and ParB homologs are involved in chromosome segregation in bacteria. ParBs participate in proper folding and initial separation of ori domains by binding to specific *parS* sites (centromere-like sequences), mainly localized in the close proximity to origin of replication *oriC*. In *P. aeruginosa* PAO1161 neither lack of *parB* gene nor modification of ten *parS*s is lethal. However, such mutants show not only defects in chromosome segregation but also growth retardation and motility dysfunctions. Moreover, in $parB_{null}$ strain expression of thousand of genes is affected.

Objectives

The aim of this study was to find all ParB binding sites in the *P. aeruginosa* genome to elucidate the role of ParB in chromosome segregation and regulation of gene expression.

Methods

DNA immunoprecipitation with anti-ParB antibodies followed by deep sequencing (ChIP-seq) analysis was performed for WT and ParB-overproducing strains of *P. aeruginosa* PAO1161. qPCR, DNA pull-down assays supported by mass spectroscopy analysis and EMSA tests were used to verify the ChIP-seq results for selected ParB binding sites.

Conclusions

ChIP-seq analysis demonstrated a specific genome-wide distribution of ParB in *P. aeruginosa.* All previously postulated *parS* sites with exception of *parS5* interacted with ParB *in vivo.* The hierarchy of *parS* sites was confirmed. Additional ParB binding sites with no or partial similarity to *parS* site consensus sequence were identified. We speculate that in *P. aeruginosa* ParB through interactions with DNA influences genome topology and modulates gene expression.

This work was supported by the National Science Centre in Poland (grant 2013/11/B/NZ2/02555).

FEMS7-3118
Physiology / Biochemistry / Molecular Microbiology

PARTITIONING PROTEIN PARB CONTROLS EXPRESSION OF GENES ADJACENT TO ITS HIGH AFFINITY BINDING SITES PARS3 AND PARS4 IN PSEUDOMONAS AERUGINOSA

A. Kawalek[1], K. Glabski[1], A.A. Bartosik[1], A. Fogtman[2], G. Jagura-Burdzy[1]
[1]Institute of Biochemistry and Biophysics- Polish Academy of Sciences, Department of Microbial Biochemistry, Warsaw, Poland
[2]Institute of Biochemistry and Biophysics- Polish Academy of Sciences, Laboratory of Microarray Analysis, Warsaw, Poland

Backgrounds

In *Pseudomonas aeruginosa* the ParABS system comprising an NTPase (ParA), a DNA-binding protein (ParB) and *parS* sequences recognized by ParB, is involved in segregation of the newly replicated chromosomes. Lack of *parB* manifests in pleiotropic phenotypic effects and leads to global changes in the transcriptome of *P. aeruginosa* cells, indicating that, directly or indirectly, ParB could play a role in regulation of gene expression.

Objectives

Here we focused on the changes in the transcriptome of *P. aeruginosa* strain exposed to a slight increase in ParB production to define the primary targets of ParB action in the cells.

Methods

Microarray-based transcriptomic analysis was performed on WT strain, its transformants with empty vector and strain carrying arabinose-inducible *araBAD*p-*parB* transcriptional fusion on a medium-copy-number plasmid. Results were verified by RT-qPCR. Chosen promoter regions were linked to *lacZ* and their activities were monitored in strains producing various amounts of ParB.

Conclusions

Increase in ParB level significantly alters the expression of 211 loci. Most notably, the mRNA level of genes adjacent to high-affinity ParB binding sites *parS1-4* close to *oriC* is reduced. Whereas *parS1* and *parS2* are intragenic to *recF* in *dnaA-dnaN-recF-gyrB* operon, *parS3* and *parS4* are located in promoter regions of operons involved in stress adaptation. In cells lacking either *parB* or functional *parS* sequences the orfs adjacent to *parS3* and *parS4* are upregulated, indicating that direct ParB interactions with *parS3* and *parS4* may repress transcription of these operons.

This work was supported by the National Science Centre in Poland (grant 2013/11/B/NZ2/02555).

FEMS7-0789
Physiology / Biochemistry / Molecular Microbiology

STUDY OF THE METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS COLONIZATION AND INFECTION IN AN ORGANOTYPIC KERATINOCYTE-FIBROBLAST CO-CULTURE MODEL

N. Barua[1], *M. Luo*[1], *M. Ip*[1]
[1]*The Chinese University Of Hong Kong, Microbiology, Hong Kong, Hong Kong S.A.R.*

Backgrounds

The difference in the array of virulence factors make some MRSA strains more virulent and capable of causing disease globally. Skin is the major site of *Staphylococcus aureus* infection and the pathogen invades across the keratinocyte barrier by modulating necrosis, pyroptosis or autophagy. An organotypic keratinocyte-fibroblast model, provides a platform to study the interactions that closely resemble the human epidermis.

Objectives

To study the adherence, internalization and the modulation of cell death by the prevalent clonal types of MRSA in a 3-D organotypic skin model.

Methods

The organotypic culture were exposed to prevalent clonal types ST239, ST45 and ST22 of healthcare-associated MRSA and ST8 (USA300), ST30 and ST59 of community-associated MRSA at log-phase containing $3.3x10^5$ CFU/ml. In addition, knockout strains of *sortase A* and *sortase B* genes of USA300 were included. Histological sections and enumeration of bacterial counts were obtained at 2hr, 16hr, 24hr and 48hr to study the adherence and internalization of the bacteria. The cell death was analyzed quantitatively by TUNEL assay and DAPI staining

Conclusions

Our study illustrates the variation in adherence, invasion and the cell death modulation among the multiple strains studied longitudinally. The MRSA clonal type ST59 showed the maximum colonization and cell death. The knockout strains of *sortase A* and *sortase B* genes of USA300 did not exhibit invasion of the organotypic skin model. The study provides an insight in the interactions between the different MRSA strains and the human skin during the establishment of the infection.

FEMS7-2197
Physiology / Biochemistry / Molecular Microbiology

AN ANTISENSE RNA CONNECTS SOS-RESPONSE WITH CELLULAR STRESS

L. Bastet[1], C. Caballero[1], M. Villanueva[1], I. Lasa[2], A. Toledo[1]
[1]Instituto de Agrobiotecnología, Laboratory of Bacterial Gene Regulation, Mutilva Baja, Spain
[2]Navarrabiomed-Universidad Pùblica de Navarra, IdiSNA, Pamplona, Spain

Backgrounds

Latest advancements in RNA-sequencing have disclosed an unexpected variety of antisense-RNAs (asRNAs) in bacteria. These non-coding RNAs are typically transcribed from the DNA strand opposite to a gene and thus present a perfect base-complementarity to the cognate mRNA. Emerging evidences indicate that asRNAs can interfere with the transcription, translation or stability of the associated mRNA. Beyond this preliminary knowledge, the physiological relevance and the regulatory mechanism of asRNAs remain elusive.

Objectives

In a previous work, we showed that several asRNAs of *Staphylococcus aureus* affect the expression level of their related gene. Among the targets, we identified the *lexA* gene, which encodes for the master regulator of the bacterial SOS-response. Herein, we aimed to further decipher the function of the *lexA* asRNA in controlling gene expression.

Methods

First, we characterized the *lexA* asRNA using Northern blot experiments and fluorescent transcriptional fusions analyzed by flow cytometry. Second, we assessed the effect of the asRNA on *lexA* expression employing time-lapse fluorescence microscopy associated to microfluidic systems. This technic allowed following the expression of fluorescent transcriptional fusions in single cells over the bacterial growth.

Conclusions

We demonstrated that the *lexA* asRNA is originated from the transcriptional read-through of the terminator of a SigmaB-controlled gene. Consequently, the expression of the asRNA is activated during the cellular stress. Microfluidic experiments suggested that the expression of *lexA* is modulated by the asRNA. Altogether, these results showed that a long complex transcript links the cellular stress with the SOS-response in *S. aureus*.

FEMS7-2588
Physiology / Biochemistry / Molecular Microbiology

CLONAL DIVERSITY OF NOSOCOMIAL MDR AND XDR ACINETOBACTER BAUMANNII ISOLATES AND PREVALENCE OF OXA GENES

T. Kula Atik[1], M. Ozyurt[1], B. Atik[2], B. Bektore[1], M.B. Selek[1], E. Yenilmez[3], O. Baylan[1]
[1]Health Sciences University- Sultan Abdulhamid Han Training and Research Hospital, Department of Medical Microbiology, Istanbul, Turkey
[2]Health Sciences University- Sultan Abdulhamid Han Training and Research Hospital, Department of Anesthesiology and Reanimation, Istanbul, Turkey
[3]Health Sciences University- Sultan Abdulhamid Han Training and Research Hospital, Department of Infectious Diseases, Istanbul, Turkey

Backgrounds

Acinetobacter baumannii is an opportunistic pathogen, which can stay alive especially in intensive care units (ICUs) for prolonged periods. Development of drug resistance, including carbapenems, causes serious problems in treatment of infections with this pathogen. Carbapenem resistance is generally caused by OXA-type carbapenemases. Pulsed field gel electrophoresis (PFGE) method, used in determination of clonal relation, is accepted as the gold standard method.

Objectives

We aimed to screen OXA-type β-lactamases and determine clonal relation in 100 distinct *A. baumannii* isolates.

Methods

All isolates are isolated from inpatients in Anesthesiology and Reanimation ICU (ARICU) and Internal Medicine ICU (IMICU) between 2012 to 2014 in our hospital. We investigated OXA-type β-lactamases via multiplex polymerase chain reaction method and clonal relation via PFGE method.

Conclusions

31 of the samples were isolated from ARICU while remaining 69 samples were from IMICU. 57% of the samples were from respiratory system. Colistin was the most effective antibiotic with 100% susceptibility rate. All isolates possessed OXA-51 gene while 92% had OXA-23 gene. During PFGE genotyping analyses, similarity coefficient was set as 80% and 19 different clusters were determined for 100 samples. The intense positivity of OXA-51 and OXA-23 genes and the proved presence of clonal relationship in *A. baumannii* isolates showed the evolution of the agent in ICU environment. While defining the hospital infection control programs to prevent the dissemination of this agent, every center should identify their OXA-type resistance profiles and common clones. We want to emphasize the importance of molecular epidemiology and related studies once more with this study.

FEMS7-1973
Physiology / Biochemistry / Molecular Microbiology

THE INFLUENCE OF DEPOSITED DIAMOND NANOPARTICLES ON LATERAL GROWTH OF BACTERIAL COLONIES

J. Beranová[1], P. Matyska Lišková[1], E. Ukraintsev[2], R. Fišer[3], O. Kofroňová[4], O. Benada[4], A. Kromka[2], I. Konopásek[1]
[1]Charles University - Faculty of Science, Department of Genetics and Microbiology, Prague 2, Czech Republic
[2]Institute of Physics of the Czech Academy of Sciences, Department of Optical Materials, Praha 6, Czech Republic
[3]Charles University - Faculty of Science, Department of Genetics and Microbiology, Prague 2, Czech Republic
[4]Institute of Microbiology of the Czech Academy of Sciences, Laboratory of Molecular Structure Characterization, Praha 4, Czech Republic

Backgrounds

Diamond nanoparticles were shown to possess bactericidal potential if applied to bacteria in suspension. However, in our previous work we also observed the influence of diamond nanoparticles (DNPs) on bacterial colony size and shape if they were applied on solid medium surface. *B. subtilis* colonies grown on DNP layer were of reduced diameter and changed shape. This effect differed for Gram-positive and Gram-negative bacterium. In this work we focused on this phenomenon in more detail.

Objectives

Colony count and colony forming ability assay is a method widely employed in testing of antibacterial potential of various materials. However, materials of nanoparticle character (such as DNPs) are deposited with bacteria on the agar plate surface during plating. We show that the nanoparticle layer can bring additional effects on bacterial colony growth in lateral direction. We observed the concentration dependent influence of DNPs on the colony area and biomass of model bacteria colonies.

Methods

Escherichia coli and *Bacillus subtilis* were chosen as representatives of Gram-negative and Gram-positive bacteria, respectively, and *Proteus mirabilis* as a model of bacterium exhibiting intensive swarming motility. We analysed the count, area and weight of bacterial colonies grown on agar surface covered with DNPs and also tested the physical characteristics of DNP-covered agar surface.

Conclusions

We conclude that DNPs function like mechanical obstacle reducing bacterial motility on the surface. This effect results in colony lateral growth suppression. We suppose that also increased hydrophobicity and roughness contributes to this motility-preventing effect of DNP layer.

FEMS7-2400
Physiology / Biochemistry / Molecular Microbiology

THE ACQUISITION OF INSERTION SEQUENCES DRIVES THE ADAPTATION OF MULTICOPY PLASMIDS TO NOVEL BACTERIAL HOSTS

C. Bernabe-Balas[1], M. Ares-Arroyo[1], A. Santos-Lopez[1], A. Hoefer[1], L. Carrilero[1], C. M. Ovejero[1], D. Thomas-Lopez[1], G. Moyano[1], J.F. Delgado-Blas[1], B. R. Matamoros[1], N. Montero[1], B. Gonzalez-Zorn[1]
[1]Faculty of Veterinary Medicine and VISAVET- Complutense University of Madrid, Animal Health, Madrid, Spain

Backgrounds

Plasmid dissemination via horizontal transfer facilitates the spread of antibiotic resistance, a critical problem worldwide. When plasmids are acquired by new bacterial hosts they need to be maintained to continue propagating their resistance genes, both horizontally and vertically. However, the mechanisms involved in plasmid adaptation are not completely understood.

Objectives

To investigate the adaptive capacity of plasmids to novel hosts.

Methods

pB1000, a multicopy plasmid from *Pasteurellaceae*, was propagated in *Escherichia coli*, where it has never been described and is rapidly lost, to explore the adaptive changes of the plasmid throughout evolution assays under antibiotic pressure. The evolved plasmids were then transformed into non-evolved *E. coli* to investigate the effects of the pB1000's adaptive modifications on its stability. The plasmid's sequence and copy number (PCN), the MIC and growth profile of the host bacteria were also analyzed, as well as the transformation efficiency both in *E. coli* and the original host.

Conclusions

Over the experimental evolutions pB1000 acquired two different insertion sequences (ISs) from the *E. coli* chromosome, both inserted into the same region of different pB1000s. Additionally, various SNPs appeared in the putative *oriV* of pB1000. Both the ISs and the SNPs triggered an increase in the stability of pB1000 in *E. coli*. While the SNP increased both the PCN and MIC, the insertion sequences reduced the biological burden of pB1000 by disrupting a specific region of the plasmid. Interestingly, both modifications impeded the retro-transformation of the original plasmid host, suggesting a non-return adaptation of pB1000 from *Pasteurellaceae* to *Enterobacteriaceae*.

FEMS7-3156
Physiology / Biochemistry / Molecular Microbiology

TERMINOMICS REVEALS EXTENSIVE PROTEOLYSIS OF CELL SURFACE PROTEINS IN A GENOME-REDUCED BACTERIAL PATHOGEN

I.J. Berry[1], V.M. Jarocki[1], J.L. Tacchi[1], B.B.A. Raymond[1], M. Widjaja[1], M.P. Padula[1], S.P. Djordjevic[1]
[1]University of Technology Sydney UTS, Faculty of Science, Ultimo, Australia

Backgrounds

Proteolytic processing profoundly affects protein function but few studies have used a systems approach to understand its role in prokaryotes. The genome-reduced pathogen, *Mycoplasma hyopneumoniae* processes members of the P97 and P102 adhesin families. It was used here as a model to study protein processing on a global scale.

Objectives

Our aim is to characterise the extent of protein processing, including methionine excision, in a genome-reduced, host-adapted pathogen. *M. hyopneumoniae* proteins that are retained on agarose resin coupled separately with different host proteins were characterised as a means to independently recover processed fragments and to determine how they differ in their binding capabilities compared with the parent. We also determined the composition of the surfaceome of *M. hyopneumoniae* and the proportion of proteins that are processed on the cell surface.

Methods

We took an untargeted, high-throughput approach to identify proteolytic events using protein mass spectrometry. Cell surface shaving and biotinylation approaches were used to confirm the surface localisation of these proteoforms and affinity chromatography bait-prey studies were used to investigate putative bait-prey interactions.

Conclusions

We identified 669 unique N-terminal peptide sequences from 164 predicted ORFs. Multiple processing events were mapped to each of 141 proteins downstream of the initiating methionine. Cleavage occurred most often with arginine in P1 and serine/threonine in P1', a pattern consistent with trypsin-like activity but other cleavage motifs were characterised. Surfaceome studies identified 159 proteins and 75 (47%) were processed extensively. Protein processing represents a mechanism to expand the function of proteins that reside on the cell surface.

FEMS7-2455
Physiology / Biochemistry / Molecular Microbiology

A NEW CLASS OF HYBRID SECRETION SYSTEM IS EMPLOYED IN PSEUDOMONAS AMYLOID BIOGENESIS

J. Berry[1], S. Rouse[1], W. Hawthrone[1], D. Chorev[2], S. Lambert[3], S. Fisentzos[1], M. Dueholm[4], D. Otzen[5], H. Bayley[2], C. Robinson[2], S. Hare[1], S. Matthews[1]
[1]Imperial College London, Life Sciences, London, United Kingdom
[2]University of Oxford, Chemistry, Oxford, United Kingdom
[3]National University of Singapore, Duke-NUS Medical School, Singapore, Singapore
[4]Aalborg University, Chemistry and Bioscience, Aalborg, Denmark
[5]Aarhus University, Molecular Biology and Genetics, Aarhus, Denmark

Backgrounds

Construction of a bacterial biofilm matrix often requires amyloid fibrils to be exported to the extracellular milieu via specialised biogenesis machineries. Amyloid production requires a dedicated multi-component secretion system through which unfolded amyloid substrates are translocated and assembled, with the most documented example being the *Escherichia coli '*Curli' system. More recently, it has come to light that *Pseudomonas* utilises a novel system for similar purposes, encoded in the single operon *fapABCDEF*, which is genetically distinct to the Curli system of *E. coli.*

Objectives

We set out to obtain structural insight into the system, starting with the outer-membrane transporter FapF. Also, we test the functional determinants of the FapC amyloid *in vivo*, and investigate the roles of other Fap components.

Methods

We use X-ray crystallography, native mass spectrometry and single channel conductance measurements to reveal the first high-resolution structural insight into the amyloid transporter FapF. We also employ mutagenesis and secretion assays to shed light on the mechanism of FapC secretion.

Conclusions

FapF forms a trimer of gated β-barrel channels, in which activation is regulated by a helical plug connected to an intriguing extended coiled-coil platform spanning the bacterial periplasm. Although FapF represents a unique type of secretion system, it shares mechanistic features with a diverse range of peptide translocation systems. Our findings highlight alternative strategies for handling and export of amyloid protein sequences, and contribute to a growing understanding of how bacteria can safely handle amyloidogenic polypeptides. The *Pseudomonas* Fap system also provides inspiration for new approaches in the control of bacterial biofilms.

FEMS7-2351
Physiology / Biochemistry / Molecular Microbiology

ADDITIONAL PROMOTERS IN THE DPS REGULATORY REGION: ACTIVATING TRANSCRIPTION AND SENSING METABOLIC CHANGES

T. Bessonova[1,2], U. Shvyreva[1], D. Gagarinskaya[1], M. Tutukina[1], O. Ozoline[1]
[1]Institute of Cell Biophysics RAS, Functional genomics and cellular stress, Pushchino, Russia
[2]Ural Federal University, Biology, Ekaterinburg, Russia

Backgrounds

Dps is a nucleoid protein well known for its protective role. Regulatory region of the *dps* gene in *E. coli* is longer than average and includes additional promoters P1, P1', P2 and P3 that stimulate transcription from Pdps. At these distal promoters, possible binding sites for proteins controlling metabolic switches, such as CRP, ExuR, GntR and SdiA, were found. This is not typical for a gene coding for nucleoid protein.

Objectives

The major aim was to study the role of metabolic regulators in mediation of *dps* expression.

Methods

Alignments were done with TCoffee and BLAST. DNA sampling results were analyzed with LC/MS-spectrometry. Mutants were prepared by "gene doctoring". Expression was measured by qRT-PCR and RNA-seq.

Conclusions

Global metabolic regulator cAMP-CRP activated *dps* in all growth conditions, while GntR acted as a repressor. Two other proteins bound to the *dps* regulatory region, ExuR and SdiA were sugar-dependent and phase-dependent dual regulators. Thus, metabolic regulators, indeed, contribute to *dps* expression. Multiple alignment showed that all *Escherichiae* and related species have Pdps and P1 promoters, but not P1', P2 and P3. As the same combination of additional *dps* promoters was found in *Dickeya dadantii*, a bacterial plant pathogen, we made a guess that these promoters were horizontally transferred to the *E. coli* genome together with their own regulation mode. At the first stage of infection, *Dickeya* starts using oligosaccharides as the main carbon source and *dps* expression becomes elevated. Additional promoters in the *dps* regulatory region may thus contribute to adaptive switch of carbon metabolism.

FEMS7-1958
Physiology / Biochemistry / Molecular Microbiology

A SENSOR MONITORING PHOSPHORYLATION OF THE CELL CYCLE RESPONSE REGULATOR CTRA IN CAULOBACTER CRESCENTUS

A. Fioravanti[1], A.A. Galizzi[1], E. Biondi[1]
[1]IMM, LCB, Marseille, France

Backgrounds

Caulobacter crescentus cells divide producing distinct cells types. The development of different cellular types is caused by the asymmetrical localization of factors before cell division. The phosphorylated response regulator CtrA represses the origin of chromosome replication and activates the expression of tens of genes that are crucial for cell cycle progression and cellular asymmetry. After the G1-S transition, in which CtrA is proteolyzed, its transcription is reactivated by GcrA in early S-phase. CtrA is then phosphorylated by the CckA/ChpT phosphorelay at the new pole of the predivisional cell. This spatial organization of CckA suggests that in predivisional cells CtrA-P levels should be higher at the swarmer new pole.

Objectives

Here we describe the development of a biosensor able to detect the phosphorylation level of CtrA in vivo by exploiting the ability of response regulators to dimerize upon phosphorylation.

Methods

The receiver domain of CtrA (CtrA-REC) was individually fused with CFP and YFP, purified and phosphorylated in vitro; the phosphorylation-dependent dimerization of the sensor was demonstrated by FRET measurements. Next we confirmed the functionality of this sensor also in vivo by expressing together both fusions in *Caulobacter* cells and measuring the FRET signals in different conditions. Finally we explore the subcellular localization of the CtrA-P levels, revealing that phosphorylation levels of CtrA in predivisional cells are higher in the proximity of the new pole.

Conclusions

The development of this biosensor indeed shows that bacterial cells are able to asymmetrically distribute phosphorylation of response regulators controlling cell cycle and the development of specific cell types.

FEMS7-1283
Physiology / Biochemistry / Molecular Microbiology

GENOME-WIDE ANALYSIS OF THE FLEQ DIRECT REGULON IN THE BIOCONTROL STRAIN PSEUDOMONAS FLUORESCENS F113

E. Blanco-Romero[1], *D. Garrido-Sanz*[1], *F. Martínez-Granero*[1], *C. Muriel*[1], *D. Durán*[1], *R. Rivilla*[1], *M. Redondo-Nieto*[1], *M. Martín*[1]
[1]*Universidad Autónoma de Madrid, Departamento de Biología- Facultad de Ciencias, Madrid, Spain*

Backgrounds

Bacterial motility plays a crucial role in competitiveness and colonization in the rhizosphere environment. Moreover, this trait is highly important in the biotechnological application and improvement of microbial inoculants employed in sustainable agriculture. FleQ appears to be the master regulator of flagella and exopolysaccharide biosynthesis in pseudomonads. Specifically, in *Pseudomonas fluorescens* F113, an interesting biocontrol and plant growth promoting bacteria, FleQ participates in the regulation of motility and biofilm formation.

Objectives

The aim of this work was to identify the genes and operons regulated by FleQ and to analyze the overlap between the FleQ and AmrZ regulons in *P. fluorescens* F113.

Methods

By using the ChIP-seq method we expected to find the DNA regions where FleQ binds to regulate gene expression. Regulation was subsequently confirmed with RT-qPCR assays in wild-type and *fleQ* mutant backgrounds.

Conclusions

ChIP-seq analysis has shown that FleQ is a global regulator in *P. fluorescens* F113 and 106 FleQ putative binding sites have been identified. Genes presumably regulated by FleQ included, as expected, flagellar and motility-related genes and others involved in adhesion and exopolysaccharide production. Surprisingly, the ChIP-seq analysis also identified iron uptake-related genes. Regulation of these genes by FleQ was verified by RT-qPCR. The results also showed that FleQ shares an important part of its direct regulon with AmrZ, a global regulator implicated in environmental adaption. Although AmrZ also regulates motility and iron uptake, the overlap occurred only with the iron-related genes, since both regulators control a different set of motility-related genes.

FEMS7-1153
Physiology / Biochemistry / Molecular Microbiology

PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF ARTHROSPIRA SP. PCC 9108 IN ORDER TO DEVELOP GENETIC ENGINEERING TOOLS

A. Blanco-Rivero[1], *M.C. Aparicio*[1], *P. Suárez-Rodríguez*[1], *F.G. Guevara*[1], *J.M. Navarro-Llorens*[1], *J. Perera*[1]
[1]*Universidad Complutense de Madrid,*
Departamento de Bioquímica y Biología Molecular- I. - Facultad de Ciencias Biológicas., Madrid, Spain

Backgrounds

The filamentous cyanobacterium known as "spirulina" or *Arthrospira platensis* is one of the most commercially important species of microalgae. Due to its high nutritional value and pharmacological and industrial applications it is extensively cultivated on a large commercial scale.

Despite its widespread use, no precise genetic manipulation tools have yet been developed to carry out important improvements in its industrial production. This is probably due to cyanobacterial defense mechanisms against foreign DNA transfer. Previous analysis performed in our laboratory show that *A. platensis* PCC9108 has 13 transcriptionally active R-M systems, which could act preventing the entry of exogenous DNA to the cell, and thus avoiding its genetic transformation**Objectives**

Before designing strategies for *Arthrospira´*s succesful transformation, two studies were aimed: studying the functionality of some of the 13 R-M systems and characterizing the growing ability of this strain under certain stressful conditions that could increase the production of certain compounds of interest.

Methods

For this work, several methods were followed: growth studies of *A. platensis* PCC9108 strain at different external pH values and different NaCl concentrations; studies of sensitivity to antibiotics; and *in vitro* endonuclease activity assays.

Conclusions

The ranges of pH values and salt concentration have been defined for the growth of *A. platensis*. Similarly, the sensibility of this cyanobacterium to several antibiotics has been evaluated. Finally, *in vitro* assays of *A. platensis* endonuclease activities have been carried out.

FEMS7-1080
Physiology / Biochemistry / Molecular Microbiology

ESCHERICHIA COLI FOF1-ATPASE ACTIVITY AND ITS COOPERATION WITH HYDROGENASE 4 (HYF) DEPEND ON GLUCOSE CONCENTRATION

S. Blbulyan[1], A. Trchounian[1]
[1]Yerevan State University, Biochemistry- Microbiology and Biotechnology, Yerevan, Armenia

Backgrounds

Escherichia coli produces dihydrogen (H_2) by hydrogenase (Hyd) enzymes during glucose fermentation. It was shown that Hyd-4 (*hyf*) operates at pH 7.5 and sensitive to the F_OF_1-ATPase's inhibitor *N,N'*-dicyclohexylcarbodiimide (DCCD).This ATPase is required for Hyd activity, and some relationships between these enzymes are suggested.

Objectives

The overall and DCCD-inhibited ATPase activity of *E. coli* BW25113 wild type (WT) and JRG3621 (*hyfB-R*) mutant membrane vesicles were studied.

Methods

Bacteria were grown anaerobically with glucose supplementation, pH 7.5. ATPase activity was determined by liberation of P_{inorg} during ATPase reaction.

Conclusions

ATPase activity of WT was 1.5-fold higher ($p \leq 0.025$) compared to mutant under 0.2% glucose fermentation. It was 1.8-fold higher compared to that of mutant upon 100 mM K^+ supplementation. Furthermore, K^+ stimulated (1.4-fold) ($p \leq 0.025$) ATPase activity in WT and 1.2-fold ($p \leq 0.02$) in the mutant. DCCD inhibited ATPase activity of both WT and mutant was 5-fold and 7-fold ($p \leq 0.02$), respectively. 0.8% glucose fermented WT showed higher ATPase activity upon K^+ free and 100mM K^+ conditions compared to 0.2% glucose fermented cells. The ATPase activity of 0.8% glucose fermented mutant was the same in dependently of K^+; DCCD inhibited markedly (~8-fold, $p \leq 0.05$) ATPase activities of investigated strains.

The F_OF_1-ATPase activity and its relationship with Hyd-4 depend on growth medium glucose concentration, which is more evident at low concentration of glucose (0.2%) and in the presence K^+. This confirms the finding that Hyd-4 is active at 0.2% glucose.

FEMS7-0704
Physiology / Biochemistry / Molecular Microbiology

BACTERIAL DRUG EFFLUX PUMP MACAB AS A NEW TARGET FOR DRUG DEVELOPMENT

L. Matrosova[1], I. Khilyas[1], T. Shirshikova[1], L. Kamaletdinova[1], Y. Danilova[1], L. Bogomolnaya[1,2]
[1]Kazan Federal Volga-Region University, Fundamental Medicine and Biology, Kazan, Russia
[2]Texas A&M University Health Science Center, Microbial Pathogenesis and Immunology, Bryan, USA

Backgrounds

Bacterial drug efflux pumps are protein membrane complexes that function to actively expulse antimicrobials from the cells. Recent data indicate that the role of drug efflux pumps is not limited to just antibiotic resistance. Macrolide-specific ABC-type drug efflux pump MacAB first identified in *E. coli* has been linked to virulence of *Salmonella enterica* serotype Typhimurium in mice. We have recently showed that MacAB efflux pump is required for protection of *Salmonella* Typhimurium against oxidative stress both *in vitro* and *in vivo*.

Objectives

Here we sought to investigate if the function of MacAB in protection against an oxidative stress is conserved across other members of *Enerobacteriaceae* family.

Methods

Here we show that MacAB is essential for survival of *Serratia marcescens* in the presence of hydrogen peroxide. We further show that *S. marcescens* Δ*macAB* mutant cells could be protected against peroxide-mediated killing by low molecular weight metabolites present in the media used to grow wild type bacteria but not in the media conditioned by growth of Δ*macAB* mutant strain. Protective effect of these metabolites was abolished by heat- or proteinase K treatment. These data indicate that the identified molecules are proteins or peptides. We are currently working on identification of MacAB substrates with anti-H_2O_2 properties.

Conclusions

Combined, our data strongly suggest that the drug efflux pump MacAB present in many Gram-negative bacteria represents an attractive target for development of new antimicrobials.

FEMS7-1006
Physiology / Biochemistry / Molecular Microbiology

DETECTION, IDENTIFICATION AND CHARACTERIZATION OF FUNGAL SPECIES IN HUMAN BREAST MILK, AND THEIR RELATIONSHIP WITH MACRONUTRIENTS AND HUMAN SOMATIC CELLS

A. Boix-Amorós[1]
[1]*Instituto de Agroquímica y Tecnología de Alimentos IATA-CSIC, Biotechnology, Paterna, Spain*

Backgrounds

Human breast milk (HBM) is considered the optimal food source for infants, providing nutrients and functional compounds unmatched by formula. Emerging evidence demonstrates the presence of bacteria in breast milk, some of which are being considered as potential probiotics to promote infant health. However, little is known about the presence of other microorganisms as yeasts and fungi, although both have been detected in gut microbiome's from infants.

Objectives

Our aim was to characterize the fungal organisms present in breast milk, their composition and load, and their relationships with macronutrients and human cells in samples from healthy lactating mothers.

Methods

Milk fungal composition was analyzed by 28S-pyrosequencing and culturing in three different media; fungal loads were estimated by qPCR calibrated by flow cytometry; and fat, protein, lactose and dry extract of milk as well as the number of human somatic cells were analyzed by spectrophotometry and cytometry.

Conclusions

Based on morphological and biochemical analysis, 21 viable fungi were isolated and identified by Sanger sequencing, most of them belonging to the species *Rhodotorula mucilaginosa* and *Candida parasilopsis*. Fungal presence was detected by specific qPCR in 89% of the samples, showing that the median fungal load was 10E5 cells/ml, 10-fold lower than previously estimated for bacteria. Pyrosequencing of 28S rDNA showed that the most common genera were *Malassezia*, *Saccharomyces* and *Candida*. There was no correlation between fungal load and somatic cells numbers, suggesting that milk fungi are not sensed as an infection by the immune system, although their origin and beneficial role should be elucidated.

FEMS7-0598
Physiology / Biochemistry / Molecular Microbiology

DEVELOPMENT OF UNIVERSAL FLUORESCENCE EXPRESSION TOOLS TO STUDY HOST-MYCOPLASMA INTERACTIONS

T. Bonnefois[1], *V. Rodrigues*[1], *P. Totté*[1], *F. Thiaucourt*[1], *L. Manso-Silvan*[1]
[1]*CIRAD, BIOS, Montferrier-sur-Lez, France*

Backgrounds

The genus *Mycoplasma* consists of Gram-positive bacteria that lack a cell wall, many of which can cause disease in humans and animals. There are no fluorescence expression systems available to monitor the dynamics of mycoplasma infections *in vivo* or *in vitro.*

Objectives

The aim of this work was to develop fluorescence expression tools for stable whole mycoplasma cell labelling and to validate their use for the study of host-mycoplasma interactions.

Methods

For this purpose, a Tn4001-derivative mini-transposon affording unmarked, stable mutagenesis in mycoplasmas was modified to allow the constitutive, high-level expression of mCherry, mKO2 and mNeonGreen. These tools were used to introduce the respective fluorescent proteins as chromosomal tags in the phylogenetically distant species *Mycoplasma mycoi*des subsp. *mycoides* and *Mycoplasma bovis*.

Conclusions

The production and characterisation of fluorescent clones were straightforward and resulted in the unprecedented observation of fluorescent colonies in the two species, with no apparent cytotoxicity. Equivalent fluorescence expression levels were quantified by flow cytometry in both species, suggesting that these tools can be broadly applied in mycoplasmas. A macrophage infection assay was performed to assess the usefulness of mNeonGreen-expressing strains for monitoring mycoplasma infections and cell invasion. The presence of fluorescent mycoplasmas inside live cells was detected and quantified by flow cytometry and corroborated by confocal microscopy, which allowed the identification of individual mycoplasmas in the cytoplasm of infected cells. The fluorescence expression tools developed in this study are suitable for host-pathogen interaction studies and offer perspectives for the functional analysis of mycoplasmas both *in vitro* and *in vivo*.

FEMS7-0201
Physiology / Biochemistry / Molecular Microbiology

EMERGENCE OF A CLONAL LINEAGE OF STREPTOCOCCUS AGALACTIAE WITH RESISTANCE TO FLUOROQUINOLONES AMONG INVASIVE ISOLATES FROM ARGENTINA RECOVERED DURING 2014-2015

B. Arias[1], V. Kovacec[1], L. Müller[1], L. Vigliarolo[2], M. Suárez[2], C. Tersigni[2], H. Lopardo[2], L. Bonofiglio[3], M. Mollerach[3]
[1]Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica, Departamento de Microbiología- Inmunología y Biotecnología- Cátedra de Microbiología, Buenos Aires, Argentina
[2]Universidad Nacional de La Plata, Facultad de Ciencias Exactas, La Plata, Argentina
[3]Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica- CONICET, Departamento de Microbiología- Inmunología y Biotecnología- Cátedra de Microbiología, Buenos Aires, Argentina

Backgrounds

Resistance to fluoroquinolones (FQ) in *Streptococcus agalactiae* (GBS) was firstly described in 2003. Currently, it has become a worldwide growing problem.

Objectives

To determine the prevalence of FQ resistance, the clonal relationship among these isolates and to analyze their epidemiological characteristics.

Methods

In the frame of a "national prospective multicenter study of *S. agalactiae* invasive infections in Argentina", 162 isolates were recovered between July 1, 2014 –June 30, 2015. A five-disc scheme of levofloxacin, ciprofloxacin, norfloxacin, ofloxacin and pefloxacin was designed to detect FQ resistant isolates. MIC of levofloxacin was performed by agar dilution. Quinolone resistance determining regions were sequenced to detect mutations. Capsular typing was done using the Strep-B Kit. Pulsed-field gel electrophoresis (PFGE) was performed to investigate the genetic relationship of GBS-FQ-resistant isolates.

Conclusions

Results: The prevalence of FQ resistance was 15.4% (25/162). All of the FQ resistant strains showed point mutations in *gyrA* and/or *parC* genes. One PFGE group (PFGE-A) accounted for 88% of the isolates (23/25) and included 13/23 isolates with identical pattern. PFGE-A clustered isolates from 9 Argentinean cities and were assigned to serotypes: Ib (14/23) and III (8/23).

Conclusions: The resistance rate (15.4 %) is higher than that found previously in our country and even in other countries of Latin America. FQ-resistant GBS isolates were recovered from different cities of Argentina and a major clone was detected.

FEMS7-0638
Physiology / Biochemistry / Molecular Microbiology

STREPTOCOCCUS PNEUMONIAE: SEROTYPE AND ANTIBIOTIC RESISTANCE IN ISOLATES RECOVERED FROM ADULTS PATIENTS IN AN ARGENTINIAN TEACHING HOSPITAL

P. Ocampo[1], V. Kovacec[2], L. Bonofiglio[3], B. Perazzi[1], M. Losada[1], S. García[1], L. Pulido[4], C. Vay[1], C. Luna[4], M. Mollerach[3], A. Famiglietti[1]
[1]Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica, Departamento de Bioquímica Clínica- Hospital de Clínicas José de San Martín, Buenos Aires, Argentina
[2]Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica, Departamento de Microbiología- Inmunología y Biotecnología- Cátedra de Microbiología, Buenos Aires, Argentina
[3]Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica- CONICET, Departamento de Microbiología- Inmunología y Biotecnología- Cátedra de Microbiología, Buenos Aires, Argentina
[4]Universidad de Buenos Aires, División Neumonología- Hospital de Clínicas José de San Martín, Buenos Aires, Argentina

Backgrounds

S. pneumoniae (Spn) is a major cause of morbi-mortality worldwide. Data regarding serotype distribution and its association with antibiotic resistance is scarce.

Objectives

The aim was to determine the serotype and antimicrobial susceptibility of Spn isolated from adult-patients that were attendant at Hospital de Clínicas, University of Buenos Aires and to evaluate the theorical coverage (TC) of PCV-13 and PPSV-23 vaccines.

Methods

Spn isolates were collected from patients >18 y.o. in two periods 2010-2012 (1P) and 2013-2014 (2P). Serotyping was performed by PCR (CDC). MIC determination was done by the BD-Phoenix-™-System.

Conclusions

Results: 86 Spn isolates were recovered, 46 belonged to 1P and 40 to 2P. All isolates except two were recovered from non-meningeal sites. 26 serotypes were identified: main 1P serotypes were S14(26%), S6(13%), S17F(13%), S19F(11%), S19A(8.7%) and S22(6.5%); from 2P were S17F(12.5%), S7F(10%), S6(7.5%) and 22F(7.5%).The TC for PCV13/PPSV23 was 59/84.6% respectively. There was a significant decrease in the TC between both periods for both vaccines (p= 0.01).

Overall, 35%(1P) and 45%(2P) of isolates were penicillin non-susceptible, most were serotypes 19A, 14, 6 and 17F. The resistance for 1P and 2P was: erythromycin (30 and 33%), clindamycin(13 and 13%), tetracycline(28 and 20%) and TMS(35 and 20%). All strains were susceptible to amoxicillin, ceftriaxone, meropenem, levofloxacin, linezolid and vancomycin.

Conclusions: A continuous surveillance program of serotypes and resistance in our Hospital is mandatory to predict treatment and monitor change in vaccine coverage since our results are different to those reported in our country.

FEMS7-2845
Physiology / Biochemistry / Molecular Microbiology

2-AMINOACRYLATE STRESS REVEALS DIVERGENCE OF SALMONELLA ENTERICA AND ESCHERICHIA COLI METABOLIC NETWORKS

A. Borchert[1], *D. Downs*[1]
[1]*University of Georgia, Microbiology, Athens, USA*

Backgrounds

The metabolic network of an organism includes the sum total of the biochemical reactions present. Roughly 80% of protein-encoding components and regulatory machinery of *Escherichia coli* K12 are shared by *Salmonella enterica* LT2. Based on this, and supported by numerous studies, it is often assumed these organisms share metabolic network structure. Multiple recent phenotypic studies suggest a need to readdress this assumption. In *S. enterica*, the enamine intermediate, 2-aminoacrylate (2AA) inactivates a number of pyridoxal 5'-phosphate(PLP)-dependent enzymes *in vivo*. The phenotypic consequence of these perturbations is determined by the metabolic network.

Objectives In this study, metabolism of 2AA and the consequences of its accumulation are investigated in *E. coli*. The results will define metabolic network differences between *S. enterica* and *E. coli*. A secondary objective is to characterize structural features of PLP-enzymes that generate, or are targeted by, 2AA.

Methods A primarily biochemical-genetic approach is used to characterize cellular components defining the 2AA stress system in both *E. coli* and *S. enterica*. Manipulation of the active site of PLP-dependent enzymes and in vitro characterization will identify residues affecting the impact of 2AA on the respective enzyme.

Conclusions

Despite conservation of relevant enzymes, *S. enterica* and *E. coli* differ in both the generation and consequence of 2AA. The findings suggest distinct structures for the metabolic network surrounding the generation and response to endogenous 2AA stress. Mechanistic investigation of traits governing 2AA generation and damage will help to refine our ability to predict the influence of 2AA in other organisms.

FEMS7-1167
Physiology / Biochemistry / Molecular Microbiology

BENZOATE DEGRADATION BY PSEUDOMONAS STUTZERI AN10

R. Bosch[1], *I. Brunet-Galmes*[2], *J.A. Christie-Oleza*[3], *J. Lalucat*[1], *J. Armengaud*[4], *B. Nogales*[1]
[1]*University of Balearic Islands UIB & IMEDEA CSIC-UIB, Microbiology, Palma de Mallorca, Spain*
[2]*University of Balearic Islands UIB, Microbiology, Palma de Mallorca, Spain*
[3]*University of Warwick, School of Life Sciences, Coventry, United Kingdom*
[4]*CEA-Marcoule, DSV/DIR/IBEB/SBTN/LBSP, Bagnols-sur-Cèze, France*

Backgrounds

Pseudomonas stutzeri AN10 (CCUG 29243) is a naphthalene degrading bacterium isolated from polluted marine sediments. Biochemical and physiological analyses demonstrated that strain AN10 was unable to use benzoate as carbon and energy source. However, its complete genome sequence revealed the presence of benzoate degradation genes (*ben*).

Objectives

In this study we have tried to clarify this discrepancy using genomic, proteomic and genetic engineering approaches.

Methods

First, we performed an in-depth genetic analysis of the AN10 *ben* genes and we observed two mutations that could be responsible for the observed phenotype: a stop codon disruption of *benR* (the transcriptional regulator) and a frameshift mutation in *catA* (catechol 1, 2-dioxygenase encoding gene). Using selective pressure we isolated strain BZ4D, an AN10-derivative able to use benzoate as carbon and energy source. Genetic analysis of BZ4D *ben* genes suggested that recovery of benzoate-degradation capability was probably due to the reversion of the two above mentioned mutations. Shotgun proteomic analysis of BZ4D grown on benzoate revealed the expression of all *ben*-encoded proteins. Finally, in order to demonstrate that both *benR* and *catA* mutations were responsible for the lack of benzoate degradation ability of strain AN10, we complemented AN10 *in trans* with both, *benR* and *catA* genes of BZ4D. As result, the recombinant AN10-derivative was able to grow on benzoate.

Conclusions

We have demonstrated that strain AN10 contained mutated forms of *benR* and *catA* genes that explained its lack of growth on benzoate, and that reversion of these mutations can be obtained by applying selective pressure.

FEMS7-3015
Physiology / Biochemistry / Molecular Microbiology

BIOCHEMICAL DIVERSITY OF CHITOSANASES BELONGING TO FAMILY GH46 OF GLYCOSIDE HYDROLASES IN THE SOIL ACTINOMYCETE KITASATOSPORA SETAE

R. Brzezinski[1], P. Viens[1], M. Zitouni[1], M. Ghinet[1]
[1]Universite de Sherbrooke, Biologie, Sherbrooke- QC, Canada

Backgrounds

Chitosan is a polysaccharide: a partly deacetylated derivative of chitin composed mainly of D-glucosamine units with β-1,4 links. Chitosan is hydrolyzed by chitosanases, belonging to several families of glycoside hydrolases (GH8, 46, 75 and 80). Many actinomycetes and bacilli produce chitosanases. However, most studies were dedicated to only one enzyme per bacterial species. The genus Kitasatospora is closely related to Streptomyces. Genome mining showed that K. setae has three GH46 chitosanase genes (csn1, csn2, csn3).

Objectives

Our aim is to characterize the biochemical diversity of the GH46 chitosanases in Kitasatospora setae.

Methods

Chitosanases were expressed in Streptomyces lividans. Chitosanase Csn2 was found in two forms: a longer polypeptide (Csn2BH) composed of a carbohydrate-binding module and a catalytic module, and a shorter form (Csn2H) composed only of the catalytic module. The activity of all four enzymes was measured against chitosans of various degrees of acetylation or chitosan complexed with natural acidic compounds: polyphosphoric acid or humic acid. Activity was compared at low temperatures. The kinetic parameters were also determined.

Conclusions

Csn1 and Csn2H were relatively similar in most tests. Csn2H had a pronounced preference for highly deacetylated chitosan and was more performant on chitosan complexed with polyphosphoric acid. Csn3 was apart from the others in all the tests: had preference for moderately acetylated chitosan, lost more activity at low temperatures, and had lower activity on complexed chitosan forms. Its specific activity was also much higher. Using 3D models for Csn1 and Csn3, we discuss how differences in structure can explain the observed biochemical diversity.

FEMS7-2324
Physiology / Biochemistry / Molecular Microbiology

STRUCTURE OF A MYCOBACTERIAL TYPE VII SECRETION SYSTEM MEMBRANE COMPLEX

C. Bunduc[1], K. Beckham[2], L. Ciccarelli[3], A. Parret[2], M. Wilmmans[2], T. Marlovits[3], W. Bitter[4], E. Houben[1]
[1]Vrije Universiteit, Molecular Microbiology, Amsterdam, The Netherlands
[2]European Molecular Biology Laboratory, EMBL, Hamburg, Germany
[3]Research Institute of Molecular Pathology, Molecular and Cellular Biology, Vienna, Austria
[4]Vrije Universiteit Medical Center, Medical Microbiology and Infection Control, Amsterdam, The Netherlands

Backgrounds

Type VII secretion (T7S) systems are used by mycobacteria to translocate a wide range of proteins across their distinct cell envelope. Pathogenic mycobacteria, including *Mycobacterium tuberculosis*, have up to five T7S systems termed ESX-1 to ESX-5, each having its own crucial role in viability and/or virulence. T7S does not resemble other known specialized secretion systems and the actual mechanism of transport is unknown. As a first step in this analysis, we have previously isolated the ESX-5 membrane complex and determined its size to be ~1.5 MDa, consisting of four membrane components, i.e. EccBCDE (ref).

Objectives

The aim of this study is to elucidate the structure and further characterize the T7S membrane complex.

Methods

We have reconstituted, isolated and analysed the ESX-5 membrane complex by negative stain single particle electron microscopy and a suite of biochemical and biophysical techniques.

Conclusions

To more easily analyse T7S systems, we reconstituted the ESX-5 system in the avirulent and fast-growing mycobacterial species *Mycobacterium smegmatis* that lacks ESX-5. The reconstituted system proved to be active and was efficiently secreting specific substrates. We subsequently purified the ESX-5 membrane complexes and analysed them through negative stain electron microscopy, resulting in the first structural images of a T7S membrane channel at 13 Å resolution. The ESX-5 complex has a six-fold symmetry and displays a marked different architecture to other bacterial secretion systems. The cytosolic domain, consisting of multiple copies of the ATPase EccC, is highly flexible. The T7S channel is not large enough to span both membranes, indicating that this core complex is located solely in the inner membrane. Finally, several lines of evidence show that EccE is a peripheral component of the transport system.

FEMS7-1414
Physiology / Biochemistry / Molecular Microbiology

ENVIRONMENTAL REGULATION OF PIA/PNAG SYNTHESIS IN STAPHYLOCOCCUS AUREUS

S. Burgui[1], *J. Valle*[1], *I. Lasa*[1]
[1]*Centro de Investigación Biomédica Navarrabiomed/ UPNA, Laboratory of Microbial Pathogenesis, Pamplona, Spain*

Backgrounds

Staphylococcus aureus is one of the leading bacteria causing nosocomial infections associated with medical devices. The difficulty of treating these infections is mainly related with the ability to form biofilms. Biofilm production protects the bacteria from both the host immune system and antibiotics. One of the main components of the *S. aureus* biofilm matrix is the intercellular exopolysaccharide adhesin PIA/PNAG, whose synthesis depends on the expression of *icaADBC*-encoded enzymes and is repressed by the *icaR* gene product. Because PIA/PNAG synthesis is expensive for bacteria, its expression needs to be tightly regulated in response to environmental conditions.

Objectives

Understand the environmental regulation of PIA/PNAG synthesis.

Methods

A collection of mutants containing a single two component (TCS) systems and different environmental conditions have been used to identify TCS responsible to connect PIA/PNAG production with environmental conditions.

Conclusions

The results revealed that synthesis of PIA/PNAG depends on ArlRS and temperature. ArlRS regulates PIA/PNAG synthesis through IcaR repressor. An ArlRS deficient strain shows higher transcriptional levels of *icaR* and consequently lower PIA/PNAG synthesis. Temperature stimulates *icaADBC* gene transcription at 28ºC and inhibits at 37ºC in an IcaR independent manner. Temperature-dependent regulation of icaADBC operon transcription occurs when *ica* operon is expressed in *S. carnosus*, a bacteria that is naturally deficient in *icaADBC* operon. These results suggested that temperature-mediated regulation of icaADBC operon depends on a regulator present both in *S. aureus* and *S. carnosus*. Alternatively, it is also possible that temperature mediated regulation depends on intrinsic structural properties of the icaADBC promoter.

FEMS7-1741
Physiology / Biochemistry / Molecular Microbiology

HYDROCARBONS DEGRADATION BY CITREICELLA AESTUARII: GENOMIC AND PHYSIOLOGICAL APPROACH

A. Busquets Bisbal[1], R. Bosch Zaragoza[2], M. Mas Llado[1], B. Nogales Fernandez[2]
[1]Universitat de les Illes Balears UIB, Microbiology, Palma de Mallorca, Spain
[2]University of the Balearic Islands UIB and IMEDEA UIB-CSIC, Microbiology, Palma de Mallorca, Spain

Backgrounds

During the course of a diversity survey in sand samples polluted after the Prestige oil tanker accident in Spain we obtained three isolates of the Roseobacter lineage in marine agar that later were shown to grow with naphthalene. Their closest relative by 16S rDNA sequence analysis was *Citreicella aestuarii* AD8^T, isolated from a tidal flat, and for which there were no reports of hydrocarbon degradation.

Objectives

The aim of the study was to determine the genomic characteristics that differentiate these four strains of *C. aestuarii*, particularly in reference to hydrocarbon catabolism.

Methods

Draft genome sequences of the strains 328, 329 and AD8T were obtained, and they were compared with the sequence of strain 357. Genomic comparison by Tetra, ANIb/ANIm and GGDC confirmed that all of them belonged to the same species. Genomes were similar in size (4.51-4.74 Mb) and mol% G+C content (64.2%-64.4%). Putative extrachromosomal elements were observed in all strains, although in variable number: 5 in strain 329, 6 in 328 and AD8T, and 8 in 357.

Conclusions

Hydrocarbon degradation genes were found in two putative plasmids present in all strains: a 183 kb-scaffold with genes for naphthalene and gentisate degradation, and a 106 kb-scaffold with homogentisate degradation genes. Growth kinetics experiments show that all strains grow with naphthalene as the sole source of carbon and energy, confirming the genomic data.The results of this study show that the ability to degrade the polyaromatic hydrocarbon naphthalene seems to be plasmid-encoded and characteristic of *C. aestuarii* strains, independently of their isolation origin

FEMS7-1044
Physiology / Biochemistry / Molecular Microbiology

CARRIAGE OF TYPE II TOXIN-ANTITOXIN SYSTEMS BY THE GROWING GROUP OF INCX PLASMIDS. A NOVEL SYSTEM REVEALED

P. Bustamante[1], *J. Iredell*[1]
[1]*Westmead Institute for Medical Research- The University of Sydney, Centre for Infectious Diseases and Microbiology, Sydney, Australia*

Backgrounds

The stable maintenance of certain plasmids in bacterial populations has contributed significantly to the current worldwide antibiotic resistance (AbR) emergency. IncX plasmids have achieved recent notoriety for their roles in transmission of resistance to carbapenem and colistin, the last-line antibiotics for Gram-negative infections. Toxin-antitoxin (TA) systems contribute to stable maintenance of many AbR plasmids, and only a few TA systems have been previously described in the IncX plasmids.

Objectives

Our main aim was to elucidate the diversity of type II TA systems carried by the IncX plasmids group and specifically to describe and characterize a novel system, TsxTA, encoded by IncX4 plasmids.

Methods

Web-tools PlasmidFinder, TAfinder and RASTA-Bacteria were used to search for IncX plasmids and TA systems, respectively. The effect of TsxTA expression on bacterial growth/physiology were analyzed by growth curves, microscopy and protein/mRNA expression measures. The contribution of TsxTA to plasmid maintenance, stress tolerance and persister cell formation, were also analysed.

Conclusions

RelE-like toxins are abundant within IncX1 and IncX4 subgroups. By contrast, the HicBA and the novel TsxTA systems are almost exclusively encoded by IncX4 plasmids. PIN, GNAT and CcdB/MazF toxins were also identified. TsxT expression causes high culture density, low cell viability, cell elongation and decrease of FtsZ cellular levels; some key cell division genes were upregulated under these conditions. TsxT increases the frequency of persister cell formation and its deletion destabilizes the plasmid.

Our results show that AbR plasmids could contain an underappreciated diversity of TA systems, some of them with novel characteristics, as the system TsxTA described here.

FEMS7-1206
Physiology / Biochemistry / Molecular Microbiology

MOLECULAR CHARACTERIZATION OF SPOT PARTNERS IN CAULOBACTER CRESCENTUS

J. Caballero-Montes[1], *K. Van Neerom*[1]
[1]*Free University of Brussels ULB, Structural Biology and Biophysics, Gosselies, Belgium*

Backgrounds

The (p)ppGpp alarmone is used by bacteria to quickly respond to environmental stress. This alarmone induces various biological modulations allowing cells to survive during the wait for a more favorable context. The a-proteobacteria *Caulobacter crescentus* exhibits an asymmetric division leading to two phenotypically different daughter cells: a sessile stalked cell and a chemotactically active motile swarmer cell that differentiates later in the sessile form. Upon nitrogen starvation, *C. crescentus* triggers (p)ppGpp accumulation, which in turn controls the cell cycle by extending the G1 swarmer phase.

Recently, a new molecular mechanism involving PTS^{Ntr} (nitrogen-related phosphotransferase system) has shown to stimulate (p)ppGpp production. PTS^{Ntr} system acts as metabolic sensor translating glutamine deprivation signal into (p)ppGpp accumulation. Once phosphorylated, the PTS^{Ntr} component $EIIA^{Ntr}$~P directly reduces the (p)ppGpp hydrolase activity of SpoT, the only RelA/SpoT homologue in *C. crescentus* responsible of the synthesis and degradation of the alarmone. The cellular (p)ppGpp level seems therefore to be increased by direct inhibitory interaction of the phosphorylated form of $EIIA^{Ntr}$ with SpoT.

Objectives

This project aims at understanding the molecular basis of bacterial stress survival mechanisms by unravelling and characterizing the interactions between the nitrogen-related phosphotransferase protein $EIIA^{Ntr}$ and the hydrolase/synthetase SpoT.

Methods

The production of high purity samples of wild-type and mutated SpoT and $EIIA^{Ntr}$ proteins will provide quantitative informations about their interactions, by Isothermal Titration Calorimetry, among others. Diffracting crystals will provide the tridimensional structure of $EIIA^{Ntr}$-SpoT complex using the power of X-Ray crystallography that will lead to new knowledges regarding $EIIA^{Ntr}$ mode of action on SpoT.

Conclusions

FEMS7-1730
Physiology / Biochemistry / Molecular Microbiology

ADAPTIVE CHANGES IN D-ALANINE-POLY(PHOSPHORIBITOL) LIGASE GENE EXPRESSION LEVEL IN LACTOBACILLUS SAKEI UNDER DIFFERENT STRESS CONDITIONS

L. Calabrone[1], *M.G. Bonomo*[1], *K. Di Tomaso*[1,2], *G. Salzano*[1]
[1]*Università degli Studi della Basilicata, Sciences, Potenza, Italy*
[2]*Università degli Studi della Basilicata, Ph.D school in Applied Biology and Environmental Safeguard, Potenza, Italy*

Backgrounds

Lactobacillus (*L.*) *sakei* is widely used as starter in the production process of fermented sausages and its growth and survival are affected by various factors such as temperature, pH and salt concentration.

Objectives

The effect of different acid, osmotic and heat stress treatments on expression level of D-alanine-poly(phosphoribitol) ligase gene was investigated by qPCR for a better knowledge of its function and role in *L. sakei* metabolism.

Methods

L. sakei cells were treated at pH 2.5 and 3.0 and 9% (w/v) NaCl for 30 min at 30°C, and at 50, 55 and 60°C. Identification and evaluation of a panel of seven reference genes for qPCR normalization was performed. The control genes were ranked according to their stability (M) values and coefficient of variation (V) using geNorm software. Once stable internal control genes were identified, the expression level of the target gene was analyzed by using the geometric mean of copy numbers as normalization factor.

Conclusions

The quantitative expression gene analysis showed that various changes in the transcription level of the gene appeared in response to different stress conditions. Particularly, a significant increase of the expression level in the samples stressed at pH 3.0 and with decreasing temperature was occurred, while a significant decrease in the presence of 9% NaCl if compared to the control was observed.

FEMS7-2222
Physiology / Biochemistry / Molecular Microbiology

EXPLORING THE TOLERANCE OF PSEUDOMONAS PUTIDA TO NON-CONVENTIONAL SALINE STRESSES

P. Calero Valdayo[1], *P.I. Nikel*[1]
[1]*Technical University of Denmark, DTU Biosustain, Kgs. Lyngby, Denmark*

Backgrounds

The soil bacterium *Pseudomonas putida* has received considerable attention in the last few years as a cell factory due, among other reasons, to its high tolerance towards stressful conditions and a wide range of compounds such as organic solvents and metal ions. However, the impact of saline stress has not been studied in depth in this bacterium. The Fluoride ion (F^-) is ubiquitous in the environment and it is known to exert antimicrobial effects (e.g. in *Escherichia coli* or *Streptococcus mutans*), which has been proposed to contribute to its anticavities activity.

Objectives

In this work, we describe the physiological effects of sodium fluoride (NaF) on the laboratory strain *P. putida* KT2440 and its reduced-genome derivatives (e.g. EM42), which have been demonstrated to display improved growth and a higher tolerance to certain stresses.

Methods

To assess the effects on *P. putida,* we compared the minimal inhibitory concentration (MIC) of F^- and Na^+ in *P. putida* KT2440 and *P. putida* EM42 to the MIC in *E. coli*, as well as toxicity curves in order to test the growth of each strain in the presence and absence of these ions. Further experimentation will provide insights into the saline stress tolerance of *P. putida* to improve the performance of this bacterium as a cell factory.

Conclusions

New tools for the assessment of intracellular F^- are currently being developed to unravel detoxification mechanisms and to highlight possible differences due to the presence of F^-.

FEMS7-1241
Physiology / Biochemistry / Molecular Microbiology

CLONAL DIVERSITY AND METHICILLIN RESISTANCE IN STAPHYLOCOCCUS EPIDERMIDIS ISOLATES FROM RUMINANTS WITH SUBCLINICAL MASTITIS

I. Camacho[1], A. Vela[2], M. Pérez-Sancho[1], L. Zamora[1], E. San Martin[1], I. Cerdá[1], J. Fernández-Garayzabal[2], L. Domínguez[1]
[1]VISAVET HEALTH SURVEILLANCE CENTRE, Identificación y Caracterización Molecular ICM, MADRID, Spain
[2]Universidad Complutense de Madrid, Sanidad Animal- Facultad de Veterinaria, Madrid, Spain

Backgrounds

Subclinical mastitis is one of the most common infectious disease found in dairy herds around the world. *Staphylococcus epidermidis* is one of the main species associated with this clinical process; however, its genetic epidemiology is poorly understood. On the other hand, resistance of this bacterium to oxacillin (methicillin) has been a matter of concern due to the possibility of transfer of resistance determinants to other bacteria.

Objectives

To improve the knowledge concerning the strain genetic diversity and prevalence of methicillin resistance of *S. epidermidis* isolates from subclinical mammary gland infections in ruminants from different farms located around Spain.

Methods

We analyzed 74 isolates from goats, sheep and cows by MLST (http://pubmlst.org/sepidermidis/). Oxacillin was used for detecting methicillin-resistant *S. epidermidis* by disk diffusion test. All isolates were additionally tested by PCR for the *mec*A and *mec*C genes (Frey et al., 2013. J Dairy Sci 96 2247–57).

Conclusions

Twenty-eight sequence types (STs) were identified. Genetic diversity 0.64, 0.35 and 0.52 among goat, sheep and cow isolates, respectively. Four STs (ST5, ST100, ST293 and ST575) represented the 51% of isolates. These STs included isolates from two (ST5 and ST575) or three animal species (ST100 and ST293). The 14.9% of isolates were resistant to methicillin. All methicillin resistant isolates harbored the *mecA* gene and were from cows. None isolate carried the *mecC* gene.

FEMS7-1244
Physiology / Biochemistry / Molecular Microbiology

IDENTIFICATION OF DESULFOVIBRIO FAIRFIELDENSIS RECOVERED FROM SWINE BY MALDI TOF MASS SPECTROMETRY

I. Camacho[1], M. Pérez-Sancho[1], M. Calderón[2], A. Casamayor[1], J. Fernández-Garayzabal[3], L. Domínguez[1], A. Vela[3], S. Vadillo[2]
[1]VISAVET HEALTH SURVEILLANCE CENTRE, Identificación y Caracterización Molecular ICM, MADRID, Spain
[2]Universidad de Extremadura, Departamento de Sanidad Animal, Badajoz, Spain
[3]Universidad Complutense de Madrid, Sanidad Animal- Facultad de Veterinaria, Madrid, Spain

Backgrounds

Desulfovibrio are sulfate-reducing anaerobic bacteria frequently isolated from the environment. However, some *Desulfovibrio* species, e.g. *D. fairfieldensis*, could be opportunistic pathogens for humans and animals. The traditional identification of *D. fairfieldensis* based on phenotypical/biochemical methods may present limitations leading to possible underdiagnoses of this bacterium.

Objectives

Evaluate the suitability of MALDI-TOF MS for the identification of *D. fairfaldensis.*

Methods

The spectra of the reference strains of *D. desulfuricans*, *D. intestinalis*, *D. vulgaris* and *D. fairfieldensis* were added to BDAL Bruker Data Base, which does not contain *Desulfovibrio* entries. Afterwards, 31 *D. fairfieldensis* isolates from mucohaemorrhagic faeces and previously identified by PCR (Loubinoux *et al*., 2002. Int J Syst Evol Microbiol 52: 1305-8) were used to assess the ability of MALDI-TOF for its identification**.** A protein extraction protocol based on ethanol-formic acid-acetonitrile was used. Spectra were acquired using a Bruker Daltonics UltrafleXtrem MALDI TOF/TOF device.

Conclusions

The MALDI ions profiles of field isolates were quite homogeneous with the presence of common ions, but with a high number of differences (m/z 3512, 4325, 4416, 4754, 5211 and 7550) with the reference strain (m/z 3641, 4263, 4407, 4776, 5392, 5643, 7282 and 8527). This result suggests a relative diversity in the protein profiles of members of this species. The inclusion of the spectra profile of one additional field isolate allowed the accurate identification of the remaining 30 *D. fairfieldensis* field isolates with a mean score value of 2.567 (2.318–2.702), suggesting good performance of MALDI TOF MS approach for identifying this species.

FEMS7-2141
Physiology / Biochemistry / Molecular Microbiology

GENETIC ANALYSIS OF PERSISTER CELL FORMATION IN PSEUDOMONAS AERUGINOSA

D. Cameron[1], *B. Mojsoska*[2], *Y. Shan*[1], *S. Molin*[2], *K. Lewis*[1]
[1]*Northeastern University, Department of Biology, Boston, USA*
[2]*Technical University of Denmark, DTU Biosustain, Kongens Lyngby, Denmark*

Backgrounds

Chronic pulmonary infection is the leading cause of mortality in patients with Cystic Fibrosis (CF). *Pseudomonas aeruginosa* is a bacterial pathogen frequently isolated from the airways of chronically infected CF patients. Multidrug tolerant persister cells contribute to the recalcitrance of infection in these cases, however the mechanisms underlying the generation of these cells in *P. aeruginosa* is poorly understood.

Objectives

In the current study, we aimed to determine the genetic determinants of persister formation in *P. aeruginosa.*

Methods

We utilized two complimentary screening approaches. First, we exposed a highly saturated transposon library of *P. aeruginosa* PAO1 to the fluoroquinolone antibiotic ofloxacin and compared the frequency of each mutant before and after treatment using transposon sequencing (Tn-seq). Secondly, we performed a high throughput screen of a collection of longitudinal genome-sequenced clinical isolates for the 'high persister' phenotype.

Conclusions

In total, 100 genes were associated with decreased ofloxacin tolerance as determined by Tn-Seq. The highest hit, *carB,* codes for the large subunit of carbamoylphosphate synthase. When challenged with antibiotics from three distinct classes, a *carB* mutant produced 5-500 fold less persister cells compared to the wild-type strain. Within longitudinal clinical *P. aeruginosa* series, up to 18 genes were correlated with the in vivo evolution of the high persister phenotype.
We have identified novel genetic pathways that affect antibiotic tolerance in *P. aeruginosa*. Understanding the precise mechanism by which these genes control the generation of drug-tolerant persisters is ongoing.

FEMS7-1216
Physiology / Biochemistry / Molecular Microbiology

THE TRANSIENT MULTIDRUG RESISTANCE PHENOTYPE OF SALMONELLA ENTERICA SWARMING CELLS IS ABOLISHED BY SOS RESPONSE ACTIVATION THROUGH CHEW TITRATION

O. Irazoki[1], S. Campoy[1], J. Barbe[1]
[1]Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, Cerdanyola del Vallès, Spain

Backgrounds

RecA protein, through its interaction with CheW, is involved in swarming motility, a rapid and coordinated multicellular migration of bacteria across a moist surface. Besides its association with virulence, during swarming, cells exhibit high-level resistance to multiple antibiotics, a phenomenon described as adaptive resistance.

Objectives

To further understand how SOS response modulates swarming, the RecA and CheW location within SOS response-activated swarming cells, the regions involved in RecA-CheW interaction and its relationship with swarming motility were studied. Further, the possible inhibitory effect of antibiotics on swarming motility and on the transient acquisition of multidrug resistance were also analyzed.

Methods

The intracellular location of these proteins within cells was analyzed by using 3D-STED microscopy. *In silico* docking studies were performed to identify the specific RecA and CheW regions associated with their interaction, that were confirmed by site-directed mutagenesis and immunoprecipitation techniques. Further, swarming ability, chemoreceptor array assembly, cell flagellation and increased antibiotic resistance phenotype in the presence of sub-lethal concentrations of several antimicrobials differing in their mechanisms of action were also analyzed.

Conclusions

Our results pointed out that the titration effect on CheW protein mediated by RecA modulates the CheW distribution within the cell when SOS response is activated, thus modulating the swarming ability. Further, and in the presence of sub-lethal concentrations of some antibiotics, this effect not only abolished swarming ability but also affects the transient multidrug-resistance phenotype acquired by swarming cells, thus revealing the potential of targeting swarming inhibition in the development of strategies to enhance the therapeutic effectiveness of antimicrobial agents.

FEMS7-2056
Physiology / Biochemistry / Molecular Microbiology

IDENTIFICATION AND CHARACTERIZATION OF RNA-BINDING PROTEINS IN STAPHYLOCOCCUS AUREUS

A. Catalán-Moreno[1], *C. Caballero*[1], *A. Toledo-Arana*[1]
[1]*Instituto de Agrobiotecnología, Laboratory of Bacterial Gene Regulation, Mutilva, Spain*

Backgrounds

In bacteria, post-transcriptional regulatory elements, such as small RNAs (sRNAs), riboswitches and RNA-binding proteins (RBP) control RNA decay, transcription elongation and translation efficiency in response to environmental changes. Although sRNAs-mediated regulation has been extensively studied, post-transcriptional regulatory mechanisms involving RBPs remain to be deciphered. RBPs are present in all kingdoms of life and can be divided in different groups depending on their protein domains: ribosomal proteins, ribonucleases or RNA chaperones.

Objectives

In order to have a better understanding of RBPs-mediated regulation, we aimed to identify and characterize the RNA chaperones and their targets using as a bacterial model *Staphylococcus aureus*, one of the most important human pathogens.

Methods

First, we performed an *in silico* analysis to identify the potential RNA chaperones present in the staphylococcal genome. Subsequently, relevant RBPs were chromosomally flagged with a 3xFLAG tag and their expression patterns analyzed by Western Blot. Finally, to identify the RNA targets recognized by RBPs *in vivo*, we performed Cross-Linking Immuno-Precipitation assays (CLIP).

Conclusions

The expression analyses revealed that the RNA chaperones, CspA, CspB, CspC, CvfB and SA_00892 are highly expressed in all the analyzed points of the growth curve of *S. aureus*. In contrast, we could not detect Hfq expression in these conditions. CLIP experiments demonstrated that the selected RBPs bind RNA *in vivo*. Preliminary results on the targets recognized by these RNA chaperones will be discussed at the meeting.

FEMS7-2422
Physiology / Biochemistry / Molecular Microbiology

MOBILIZATION MECHANISM OF PATHOGENICITY ISLANDS BY ENDOGENOUS PHAGES IN STAPHYLOCOCCUS AUREUS

M. Cervera Alamar[1], *K. Guzmán*[1], *M. Žiemytė*[1], *M.Á. Tormo Mas*[1]
[1]*Hospital Universitario la Fe, Infección Grave, Valencia, Spain*

Backgrounds

Staphylococcus aureus is a pathogen that is able to cause a variety of community and hospital acquired diseases. The broad ranges of infections caused by *S.aureus* are related to a number of virulence factors. Most of these virulence factors are encoded in mobile genetic elements (MGE). We concentrate in *S. aureus* pathogenicity islands (SaPIs) which are controlled by the master repressor (Stl). This repressor is activated by helper phages, which encode specific inductor proteins. Binding of Stl and the inductor induces the SaPI excision, replication and packaging cycle. These events allow transference of SaPIs spreading virulence factors among bacteria.

Objectives

To detect strains with endogenous phages capable to mobilize its own SaPIs and to identify the specific inductor protein

Methods

.

Conclusions

We tested SaPIs mobilization of 15 sequenced clinical *S. aureus* strains. Five strains were induced and mobilized by its endogenous phages. We focused in the regulation module of two strains whose SaPIs present the same Stl. We identified the endogenous phage responsible of SaPI induction and its specific inductor protein. This protein corresponds to a hypothetical protein with a conserved domain. Mutant of this protein was not able to induce and mobilize the SaPI. We also demonstrated the binding Stl-inductor and the existence of allelic variants of this protein codified in other phages with different affinities for Stl. This confirms that this mechanism is general for others strains. In the future we would like to characterize the protein structure, the binding motif to Stl and to determine the function in the phage.

FEMS7-0346
Physiology / Biochemistry / Molecular Microbiology

EPSTEIN-BARR VIRUS-ENCODED MIR-BART5-5P UPREGULATES PD-L1 THROUGH PIAS3/PSTAT3 MODULATION IN GASTRIC CARCINOMA CELLS

D.H. Kim[1], C.J. Yoon[1], J.S. Kim[1], S. Park[2], E. Choi[2], J.H. Woo[1], M.S. Chang[2]
[1]Asan Institute for Life Sciences- University of Ulsan College of Medicine, infectious diseases, Seoul, Republic of Korea
[2]Seoul National University College of Medicine- Boramae Hospital, Pathology, Seoul, Republic of Korea

Backgrounds

Epstein-Barr virus (EBV) infection is detected in a subset of gastric carcinomas, about 5~10% of the total gastric carcinoma cases. MicroRNAs (miRs) are 22±2 nucleotide non-coding RNAs, and regulate various functions in cells. The function of EBV-encoded microRNA-BART5 (miR-BART5) has been elucidating.

Objectives

The aim of the present study was to investigate target proteins for miR-BART5 and alteration of cellular proteins and biologic properties by miR-BART5 in gastric carcinoma cells.

Methods

The miR-BART5-5p mimicker or miR-BART5-5p-stable transfectants in EBV-negative gastric carcinoma cells was constructed. Apoptosis was quantified with the annexin V-fluorescein isothiocyanate (FITC) apoptosis kit on a FACScan flow cytometer. For cell proliferation assay, ‘cell counting kit-8’ (CCK-8) was used. The miR and protein levels were measured using Taqman RT-PCR and western blot, respectively. To search a direct target of miR-BART5-5p, bioinformatics analysis and 3'UTR luciferase activity assay were performed. As a result, there were no significant differences in proliferation or apoptosis between miR-BART5-5p-expressing gastric carcinoma cells and miR-control-transfected cells. PIAS3 protein expression was significantly lower in two cell lines of naturally EBV-infected gastric carcinoma than in three cell lines of EBV-negative gastric carcinomas**.** PIAS3 protein expression was reduced in miR-BART5-5p-transfected EBV-negative gastric carcinoma cells. Additionally, PIAS3 3′UTR reporter activity was suppressed in miR-BART5-5p-expressing gastric carcinoma cells. As a downstream of PIAS3, ‘phospho-signal transducer and activator of transcription 3’ (pSTAT3) was considered. The pSTAT3 increased in miR-BART5-5p expressing gastric carcinoma cells, which was dependent upon PIAS3, Interestingly, ‘programmed cell death ligand 1’ (PD-L1), one of proteins associated with immune regulation, increased in miR-BART5-5p expressing gastric carcinoma cells in a pSTAT3-dependent manner.

Conclusions

PIAS3 is a direct target of miR-BART5-5p in gastric carcinoma cells. PIAS3 decrease leads to pSTAT3 increase, and PD-L1 was upregulated in pSTAT3-dependent manner. These findings may provide a clue of the immunologic modulation mechanism in EBV-infected gastric carcinoma.

FEMS7-0744
Physiology / Biochemistry / Molecular Microbiology

STRUCTURAL BASIS OF MEMBRANE PORE-FORMATION MECHANISM OF BACTERIAL PORE-FORMING TOXINS

N. Kundu[1], R. Kathuria[1], K. Chattopadhyay[1]
[1]Indian Institute of Science Education and Research Mohali, Department of Biological Sciences, Mohali, India

Backgrounds

Pore-forming toxins (PFTs) represent a unique class of protein toxins that are ubiquitously found in wide array of organisms ranging from bacteria to human. PFTs act by punching holes in the membranes of their target cells, thereby leading to killing of the target cells. PFTs act as the major virulence factors of many pathogenic bacteria. Indeed, one third of the bacterial protein toxins belong to the PFT family. In their generalized mode of action, PFTs are secreted by the pathogenic bacteria, which in contact with their target eukaryotic cells generate the transmembrane pores, thus leading to membrane-permeabilization, and killing of the target host cells.

Objectives

In spite of sharing an overall general mode of action, individual members of the bacterial PFT family differ from each other in the intricate details of their mechanism of action. Therefore, characterization of the structure-function mechanism of each of the individual bacterial PFTs is essential in terms of elucidating their mode of action.

Methods

In our study, we have explored the multiple facets of the structural mechanisms associated with some of the archetypical bacterial PFTs, and examined their functional implications. Specifically, we have explored the functional implications of specific structural motifs/domains present in the PFTs. We have explored the mechanisms of membrane pore-formation employed by the PFTs, and examined the implications of specific membrane components in regulating such processes.

Conclusions

Results obtained from our study have provided novel insights regarding the structural basis associated with the mode of action of some of the prominent bacterial PFTs.

FEMS7-0623
Physiology / Biochemistry / Molecular Microbiology

IDENTIFICATION AND PRELIMINARY CHARACTERIZATION OF A XYLANASE GENE FROM A NOVEL SPECIES OF ANTARCTIC FUNGUS

R. Chavez[1], C. Gil-Duran[1], C. Ravanal[2], I. Vaca[3]
[1]Universidad de Santiago de Chile, Departamento de Biologia, Santiago, Chile
[2]Universidad de Chile, Departamento de Ingenieria Quimica y Biotecnologia, Santiago, Chile
[3]Universidad de Chile, Departamento de Quimica, Santiago, Chile

Backgrounds

Xylan is one of the main hemicellulose polysaccharide in plant cell walls. The biodegradation of xylan requires a set of enzymes called xylanases. To date, most xylanolytic fungi studied have been isolated from mesophilic and thermophilic terrestrial environments. On the contrary, very little is known about xylanolytic activities in Antarctic marine fungi. In our laboratory, we recently isolated a strain of the Antarctic marine fungus *Cladosporium* sp. In previous analyses, this fungus showed high xylanolytic activity at low temperature.

Objectives

In the present work, we advance in the phylogenetic identification of *Cladosporium* sp. In addition, we describe preliminary results about the characterization of a xylanase gene from this fungus, and its expression.

Methods

The phylogenetic position of *Cladosporium* sp. within the currently known species of the genus *Cladosporium* was determined by using three suitable molecular markers. On the other hand, part of a gene (750 base pairs) encoding for an endoxylanase, was isolated by PCR. Finally, the expression of this gene in the presence of xylan as carbon source at 15 °C was assessed by RT-PCR.

Conclusions

Our results indicate that *Cladosporium* sp. is phylogenetically different from all other known species of the genus and likely, it is a new species not described yet. On the other hand, the endoxylanase gene from this fungus has maximal expression at day 2 of culture at 15 °C. In summary, the Antarctic fungus *Cladosporium* sp. contains a gene encoding for a xylanase that is expressed at low temperatures. Work supported by grant INACH RG_03-14 and DICYT-USACH.

FEMS7-3182
Physiology / Biochemistry / Molecular Microbiology

ERK AND AMPK SIGNALING PATHWAYS INVOLVE IN APICAL SOLUTION-INDUCED DOWNREGULATION OF ION TRANSPORT IN CULTURED PIG TRACHEAL EPITHELIA

W. Xu[1,2], W.N.F. Li[1,2], J.H. Chen[1,2]
[1]University of Hong Kong, School of Biomedical Sciences, Hong Kong, Hong Kong S.A.R.
[2]HKU Shenzhen Institute of Research and Innovation, ., Shenzhen, China

Backgrounds

Respiratory epithelium controls transepithelial ion and fluid transport for maintaining homeostasis of airway surface.

Objectives

Whether the epithelia could sense and respond to surface fluid changes is vastly unknown.

Methods

To test this hypothesis, we gently washed and then covered the apical surface of cultured pig tracheal epithelia with a thin layer of the Krebs solution, followed by examining alterations in the transepithelial short-circuit currents (Isc) at 1 to 24 hr after the treatment.

Conclusions

Our data demonstrate that either Isc at basal condition (Isc_{-Basal}) or reduction of Isc by adding the epithelial Na^+ channel (ENaC) inhibitor amiloride (ΔIsc_{-Amil}) was largely decreased at 4, 7 and 10 hr after apical application of the Krebs solution, compared to that of the control epithelia with no treatment. Similarly, the study on stimulation of the cystic fibrosis transmembrane conductance regulator (CFTR) activity by forskolin and IBMX found that drug-induced increase in Isc ($\Delta Isc_{-F\&I}$) were also moderately reduced at 7 hr after the treatment. These data indicate that the apical Krebs solution caused transient reductions in ENaC- and CFTR-mediated ion transport of tracheal epithelia. In addition, epithelia basolaterally pretreated with the ERK inhibitor U0126 or the AMPK inhibitor compound C all largely attenuated the inhibition on Isc_{-Basal} and ΔIsc_{-Amil} by the apical Krebs solution. These data suggest that fluid challenge on the luminal surface of the tracheal epithelia may reduce the ENaC activity in the apical membrane, partly due to activation of the ERK and AMPK signaling pathways.

FEMS7-0376
Physiology / Biochemistry / Molecular Microbiology

EMRR, A MARR-REGULATOR FOR ALCOHOL METABOLISM AND ORGANIC PEROXIDE-SENSING IN ACINETOBACTOR BAUMANNII.

S.J. Chen[1], *G.H. Lin*[1]
[1]*Tzu Chi University, Master Program in Microbiology and Immunology, Hualien, Taiwan R.O.C.*

Backgrounds

Acinetobacter baumannii, an aerobic gram-negative bacterium, is one of the most important nosocomial pathogen. Previous study showed that low-level ethanol-exposure induces the expression of MarR(Multiple Antibiotic Resistant Regulator) regulator in *A. baumannii*. This MarR regulator was named as Ethanol-related MarR regulator (EmrR) here. *In silico* analysis, OhrR(Organic hydroperoxide resistance), the MarR protein that can regulated oxidative stress in *Xanthomonas campestris*, is 56.3% identity to EmrR. OhrR regulated *ohr* which encode a thiol-dependent peroxidase directly catalyze the reduction of organic peroxidases to less toxic organic alcohol in *Xanthomonas campestris*.

Objectives

Investigate the function of EmrR(Ethanol-related MarR regulator) for alcohol metabolism and organic peroxide-sensing in *Acinetobactor baumannii.*

Methods

In order to prove whether *emrR* was related to ethanol metabolism in *A. baumannii*. *emrR* mutant its growth condition is compared to wild type with cultivation in difference concentration of alcohol as the sole carbon source. Promoter activity of *adh* (alcohol dehydrogenase) in ethanol condition was determined by GFP assay. The EMSA(Electrophoretic Mobility Shift Assay) was performed to know which promoters were bound by EmrR directly. Disk assay was determined to know *emrR* mutant its growth condition is compared to wild type with cultivation in difference concentration of organic peroxide.

Conclusions

Results indicated ADH4 expression was repressed by EmrR in *A. baumannii*. An *emrR* mutant is more sensitive to *tert*-butyl hydroperoxide than wild type, indicating that EmrR mediates the defensive system against organic peroxide.

FEMS7-0210
Physiology / Biochemistry / Molecular Microbiology

BISMUTH-BASED DRUGS INHIBIT- PORPHYROMONAS GINGIVALIS: PROTEOMIC/TRANSCRIPTOMIC PROFILING AND MOLECULAR MECHANISMS

T. Cheng[1], Y.T. Lai[2], Y. Yang[2], H. Sun[2], L. Jin[1]
[1]The University of Hong Kong, Faculty of Dentistry, Hong Kong, Hong Kong S.A.R.
[2]The University of Hong Kong, Department of Chemistry, Hong Kong, Hong Kong S.A.R.

Backgrounds

Porphyromonas gingivalis is a 'keystone' pathogen for the highly common periodontal disease worldwide.

Objectives

This study aimed to investigate the protein targets and effects of bismuth-based drugs on the gene expression of *P. gingivalis*, through metal-based proteomics and RNA seq & Real-time PCR.

Methods

The minimal inhibition concentration of ranitidine bismuth citrate (RBC) for *P. gingivalis* was determined after 48-hour incubation with series-diluted RBC and other antimicrobials. Fluorescence-labeling-based 2D-PAGE was performed on *P. gingivalis* cell lysates with a fluorescence probe of Bi^{3+}-TRACER. The identified proteins were selected and cloned, and subsequently recombinant proteins were overexpressed and purified for activity determination. The effects of bismuth on mRNA expression profile of *P. gingivalis* were analyzed by RNA seq, and selected genes were further evaluated by Real-time PCR.

Conclusions

It was shown that bismuth could inhibit *P. gingivalis* growth, and enhance the bacterial sensitivity to antimicrobials (e.g. metronidazole and *chlorhexidine*). Multiple protein spots on the 2D-PAGE of *P. gingivalis* lysates were lit up by Bi^{3+}-TRACER after UV-radiation, and dozens of potential bismuth-associating proteins were identified by peptide mass fingerprinting on these lit-up spots. Notably, bismuth could partially or even completely inactivate these selected proteins. Whole-genome RNA seq revealed that the expression of substantial amounts of genes with various functions was markedly affected during the first hour of bismuth treatment which was selectively verified by Real-time PCR. This pioneering study indicates that *P. gingivalis* undergoes extensive influences by bismuth which leads to bacterial inhibition.

FEMS7-0313
Physiology / Biochemistry / Molecular Microbiology

DETECTION AND QUANTIFICATION OF DIFFERENTIALLY CULTURABLE TUBERCLE BACTERIA IN SPUTUM FROM PATIENTS WITH TUBERCULOSIS

M. Chengalroyen[1], G. Beukes[1], B. Gordhan[1], E. Streicher[2], G. Churchyard[3], R. Hafner[4], N. Martinson[5], K. Otwombe[5], B. Kana[6]
[1]Centre of Excellence for Biomedical TB Research, Pathology, Johannesburg, South Africa
[2]MRC Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Cape Town, South Africa
[3]The Aurum Institute, Aurum, Johannesburg, South Africa
[4]Tuberculosis Clinical Research Team, Department of Health and Human Services-, Bethesda, USA
[5]Perinatal HIV Research Unit, Health Science, Johannesburg, South Africa
[6]Center of Excellence for Biomedical TB Research, Pathology, Johannesburg, South Africa

Backgrounds

Recent studies suggest that baseline tuberculous sputum comprises a mixture of routinely culturable and differentially culturable tubercle bacteria (DCTB). The latter seems to be drug tolerant and dependent on resuscitation-promoting factors (Rpfs) for growth.

Objectives

To further explore this, we assessed sputum from patients with tuberculosis for DCTB and studied the impact of exogenous Rpf-containing or deficient culture filtrate (CF) supplementation *ex vivo*.

Methods

Sputum samples from adults with tuberculosis and either HIV-1 or no HIV-1 were used for most probable number (MPN) assays supplemented with CF and Rpf-deficient CF, to detect CF-dependent and Rpf-independent DCTB, respectively.

Conclusions

In 110 individuals, 19.1% harbored CF-dependent DCTB. Furthermore, 11.8% yielded Rpf-independent DCTB. In addition, 53.6% displayed both CF-dependent and Rpf-independent DCTB, 1.8% carried CF-independent DCTB, and 13.6% had no DCTB. Sputum from individuals without HIV-1 yielded higher CF-supplemented MPN counts compared with their counterparts. Furthermore, individuals with HIV-1 with CD4 counts greater than 200 cells/mm3 displayed higher CF-supplemented MPN counts compared with participants with HIV-1 with CD4 counts less than 200 cells/mm3. Notably, CF supplementation allowed for detection of mycobacteria in 34 patients with no culturable bacteria on solid media. Additionally, the use of CF enhanced detection of sputum smear–negative individuals.These observations demonstrate a novel Rpf-independent DCTB population in sputum and reveal that reduced host immunity is associated with lower prevalence of CF-responsive bacteria. Quantification of DCTB in standard TB diagnosis would be beneficial because these organisms provide a putative biomarker to monitor treatment response and risk of disease recurrence.

FEMS7-3161
Physiology / Biochemistry / Molecular Microbiology

BIOCONVERSION OF ALGAL BIOMASS: METABOLIC POTENTIAL OF ZOBELLIA AMURSKYENSIS

N. Chernysheva[1], A. Stenkova[1], O. Nedashkovskaya[2], M. Isaeva[1]
[1]G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Laboratory of marine biochemistry, Vladivostok, Russia
[2]G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Laboratory of microbiology, Vladivostok, Russia

Backgrounds

The algal sulfated and carboxylated polysaccharides have been extensively studied for their variable biological activities (antitumor, anticoagulant, and antiviral). Marine polysaccharides are complex decorated, therefore they are less bioavailable and their study tightly associates with the polysaccharide degrading/modifying enzymes. For algal biomass converting the investigation of alga-associated bacteria and their enzymes has a great value. Marine flavobacteria possess a remarkable metabolic potential for effective utilization of seaweeds. Very famous of them is *Zobellia galactinovorans*, which harbors an enormous number of carbohydrate active enzymes.

Objectives

The objective was sequencing and genomic analysis of another *Zobellia* species isolated from a seawater sample collected in Amursky Bay, Sea of Japan.

Methods

Draft genome of the *Zobellia amurskyensis* 3526^T was obtained using the Roche/454 pyrosequencing technology on the GS Junior. Assembly, automatic and manual annotations of the genome were performed via Newbler v3.0 software, RAST annotation server and BLAST search on the Protein Database, Swiss-Prot, COG and KEGG databases. Genomic analysis was focused on genes encoding carbohydrate-active enzymes using CAZy database.

Conclusions

We revealed that the genome encodes a great number of carbohydrate-active enzymes covering many of known enzyme classes according to CAZy nomenclature. Additionally, the genome is enriched with sulfatase genes. Many of the detected genes are arranged in Polysaccharide Utilization Loci. The enzymatic repertoire enables *Z. amurskyensis* to decompose green, red and brown algal biomass, and participates in marine organic carbon cycling.

This work was supported by the Program for Basic Research FEB RAS “Far East”, project №15-I-5-014.

FEMS7-0447
Physiology / Biochemistry / Molecular Microbiology

ELUCIDATION OF NOVEL ROLE OF ESCHERICHIA COLI BIPA EXPRESSED AT LOW TEMPERATURE

E. Choi[1], *M. Lim*[1], *H. Jihwan*[1]
[1]*Pusan National University, Microbiology, Busan, Republic of Korea*

Backgrounds

BipA is a conserved ribosome-associated GTPase and shares structural similarity with other translational GTPases such as IF-2, EF-Tu, and EF-G. Its binding site on the ribosome appears to overlap with that of those translational GTPases. Mutation in *bipA* causes a variety of phenotypes, including cold sensitivity, antibiotics sensitivity, and decreased pathogenicity, implying that BipA may participate in diverse cellular processes by regulation of translation. According to the recent studies, *bipA*-deletion strains show ribosome assembly defect at low temperature, suggesting that BipA may be involved in ribosome assembly. Despite such extensive research on BipA, the exact function of BipA is still unknown.

Objectives

Our ultimate objectives are to find the reason why growth and ribosome assembly defects were observed only at low temperature and to elucidate precise role of BipA at low temperature in *Escherichia coli*.

Methods

We analyzed ribosomal protein composition of pre-50S particles accumulated in *bipA*-deletion strain at 20°C by sucrose gradient sedimentation and SDS-PAGE. Then, we carried out qRT-PCR to examine the expression pattern of *bipA* at various temperatures, and did mutational analysis of the BipA protein. Lastly, we tested chaperone activity of BipA by using denatured green fluorescence protein (GFP).

Conclusions

Our results showed that BipA is expressed under low temperature condition and deletion of *bipA* causes accumulation of pre-50S particles lacking L6 at 20°C. In addition, BipA possesses chaperone activity.. These results demonstrate that BipA is a novel ribosome-associated translational GTPase which is induced at low temperature and facilitates the folding of ribosomal protein and ribosomal subunit.

FEMS7-0255
Physiology / Biochemistry / Molecular Microbiology

PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A LACCASE FROM COPRINUS COMATUS

H. Choi[1], *E. Park*[2]
[1]*Kangwon National University, Biochemistry, Chuncheon-Si- Gangwon-Do, Republic of Korea*
[2]*Kangwon National University, Biochemistry, Chunchon, Republic of Korea*

Backgrounds

Laccases (benzenediole: oxygen oxidoreductase, EC 1.10.3.2) are multicopper blue oxidases, and there have been many reports that fungal laccases are involved in fungal development, pigment generation and pathogenicity of plant/animal infecting fungi. We have worked many laccase isozymes from several mushroom-forming fungi, and their biochemical characteristics were determined. We have also analyzed the enzyme applications for the degradation of recalcitrant chemicals such as dyes, endocrine-disrupting compounds and explosives.

Objectives

Coprinus comatus, an inky cap, synthesizes and secretes laccase in YEPD (yeast extract 10 g, peptone 20 g, dextrose 20 g, 1 L d-H_2O) liquid medium at neutral pH. The culture broth turned black after day 3, and laccase synthesizes this black melanin. We have purified a laccase from a liquid culture of this fungus, and its biochemical characteristic and its dye-decolorization activity were examined.

Methods

Coprinus comatus were grown in YEPD liquid medium (250 ml in 1 L flask) for 3 days in a shaking incubator, and the culture supernatants were collected for the enzyme source. A laccase was purified through DEAE-Sapharose ion-exchange chromatography and polyacrylamide preparative gel electrophoresis.

Conclusions

The biochemical parameters were as follows: optimum temperature was 25ºC and optimum pH was 4.3 when *o*-tolidine was used as a substrate, and its estimated molecular weight was 52 KDa. We have used this enzyme for the degradation of PolyR-478 and Remazol Brilliant Blue R dyes at 25ºC, and the enzyme treatment resulted in decolorization of both dyes. This enzyme can be used for the industrial dye removal

FEMS7-1558
Physiology / Biochemistry / Molecular Microbiology

CONTRIBUTION OF LON AND CLPP PROTEASES OF PANTOEA ANANATIS TO VIRULENCE

O. Choi[1], B. Kang[2], J. Park[2], Y. Lee[2], J. Kim[3]
[1]Gyeongsang National University, Institue of Agriculture & Life Science, Jinju, Republic of Korea
[2]Gyeongsang National University, Division of Applied Life Science BK21 Plus, Jinju, Republic of Korea
[3]Gyeongsang National University, Institue of Agriculture & Life Science- Division of Applied Life Science BK21 Plus, Jinju, Republic of Korea

Backgrounds

The ATP-dependent proteases Lon and ClpP (Clp proteolytic subunit) are responsible for the turnover of misfolded proteins and the degradation of regulatory proteins in bacterial cells. γ-proteobacterium *Pantoea ananatis* PA13, our model bacterium, causes bacterial rice sheath rot and onion center rot diseases.

Objectives

The objective of this study was to assess the contribution of Lon and ClpP proteases of *P. ananatis* to virulence.

Methods

Using *Mariner* transposon mutant screening, we isolated *lon* and *clpP* mutants, exhibiting the deficiency in swimming motility and virulence in rice. We constructed mutants in the *lon* and *clpP* genes of *P. ananatis* PA13 by SacB-dependent manner and found that both mutants were impaired in growth at 28°C, swimming motility, and virulence.

Conclusions

The *lon* mutant of *P. ananatis* displayed a severe defect in colony morphology. The growth of the *clpP* mutant of *P. ananatis* was impaired at high temperature, a condition known to increase the level of misfolded protein. Further characterization of the *lon* mutant revealed enhanced autoagglutination. All phenotype deficiencies of both mutants were recovered by genetic complementation with intact *lon* and *clpP* (pOR84; pBBR1MCS-5::*lon* and pOR78; pBBR1MCS-5::*clpP*, respectively). These data suggested that the proteases may contribute to the virulence of *P. ananatis*.

FEMS7-0079
Physiology / Biochemistry / Molecular Microbiology

MOLECULAR CHARACTERISTICS OF HLYU, A TRANSCRIPTIONAL REGULATOR OF VIBRIO VULNIFICUS VIRULENCE GENES

S.H. Choi[1], Z.W. Lee[1], Y.J. Bang[2], K.K. Jang[1]
[1]Seoul National University, Department of Agricultural Biotechnology, Seoul, Republic of Korea
[2]The University of Texas Southwestern Medical Center, Department of Immunology, Dallas, USA

Backgrounds

An opportunistic pathogen *Vibrio vulnificus* produces a large pore-forming toxin, RtxA, which triggers necrotic and apoptotic cell death. Previous studies reported that HlyU upregulates *rtxA* expression by direct binding to the promoter region of the *rtxHCA* operon.

Objectives

As a transcriptional regulator, HlyU was further characterized, and the mechanism of HlyU in *rtxA* regulation was elucidated.

Methods

The *hlyU* mutant was constructed, and the transcriptomic profiles of the wild type and the *hlyU* mutant were analyzed by RNA-sequencing. In addition to *rtxA*, genes encoding virulence factors such as hemolysin and phospholipase were down-regulated in the *hlyU* mutant, implying that HlyU contributes to the *V. vulnificus* pathogenesis by regulating various virulence genes. Furthermore, the levels of *rtxA* and *hlyU* transcripts in *V. vulnificus* cells grown in different conditions were determined. Induction of the *rtxA* expression occurred in the wild-type cells either exposed to the INT-407 human epithelial cells or grown under anaerobic conditions, but not in the *hlyU* mutant, indicating that HlyU mediated the induction. Since the levels of *hlyU* transcript in the wild-type cells were not significantly changed in those conditions, the *rtxA* activation might be attributed to altered activity rather than amounts of HlyU. To better understand this activity alteration of HlyU, C30, C96, and M87, which were predicted as essential amino acids from structural analysis, were mutated by site-directed mutagenesis. Among the mutants, *V. vulnificus* expressing HlyU-C30S showed a decreased *rtxA* level and cytotoxicity toward the HT-29 MTX cells than the wild type, suggesting that C30 is a critical residue of HlyU for *rtxA* activation.

Conclusions

Taken together, HlyU might act as a global regulator for the various virulence genes and induce *rtxA* under host environment by altering its activity.
This research was supported by a grant (14162MFDS172) from Ministry of Food and Drug Safety in 2017.

FEMS7-0247
Physiology / Biochemistry / Molecular Microbiology

EVALUATION OF REFERENCE GENES FOR GENE EXPRESSION ANALYSIS USING QUANTITATIVE RT-PCR IN MICROSPORUM CANIS

A. Ciesielska[1], *P. Staczek*[1]
[1]*University of Lodz, Department of Microbial Genetics, Lodz, Poland*

Backgrounds

The selection of reference genes used for data normalization to quantify gene expression by qRT-PCR is a crucial step of this technique. Moreover, little information regarding such genes is available for gene expression analyses in dermatophytes.

Objectives

We investigated the suitability of nine candidate reference genes in isolates of *Microsporum canis* subjected to different environmental stimuli typical for the stage of host infection such as different carbon sources, pH shifts, growth phase transition.

Methods

The stability of these genes was determined by NormFinder, geNorm and BestKeeper software.

Conclusions

To the best of our knowledge this is the first report on selection of reference genes for qRT-PCR data normalization in *Microsporum canis* and the results of these studies should permit further analysis of *Microsporum canis* gene expression under several conditions.

FEMS7-0251
Physiology / Biochemistry / Molecular Microbiology

CHARACTERIZATION OF THE LIPOPROTEIN FROM THE CPR RESISTANCE MACHINERY OF CLOSTRIDIUM DIFFICILE

R. Clemens[1], *S.H.J. Smits*[1]
[1]*Heinrich-Heine-University- Duesseldorf, Institute of Biochemistry, Duesseldorf, Germany*

Backgrounds

The Gram-positive bacterium *Clostridium difficile* is a human pathogen that causes chronic intestinal disease. This spore forming anaerobe bacterium is resistant against antibiotics. Therefore a special need is to obtain new antibiotics to cure the diseases associated with *C. difficile*. Here, lantibiotics which are small antimicrobial peptides due to their high antmicrobial activities interesting candidates. .

However, within the *C. difficile* genome a protein machinery generating a lantibiotic resistance is encoded, the so called Cpr system (McBride and Sonenshein 2011). Upstream of this system a lipoprotein is localized encoding *Cd*Lipo, similar to other lantibiotic resistance systems (Khosa et al. (2016)). *Cd*Lipo however, is uncharacterized till date. The understanding of the molecular mechanism of this *Cd*Lipo protein would allow to synthesise an effective compound circumventing the occurring lantibiotic resistance.

Objectives

The goal of this study is to characterize the *Cd*Lipo.

Methods

To characterize *Cd*Lipo, we express in *Escherichia coli* and purify the lipoprotein with affinity and size exclusion chromatography. To get some information about its function we do co-elution studies with the purified *Cd*Lipo and several potential interaction partners for example the model lantibiotic nisin. In addition we do structural studies to obtain the first glimpse of this protein using a combination of SAXS, NMR and crystallography measurements.

Conclusions

Here we present our initial structural as well as functional results of the *Cd*Lipo protein using these *in vivo* and *in vitro* results and clarify its role in lantibiotic resistance.

FEMS7-2601
Physiology / Biochemistry / Molecular Microbiology

NEW INSIGHTS INTO THE ASSEMBLY OF ESCHERICHIA COLI OUTER MEMBRANE LIPOPROTEINS

S.H. Cho[1], A. Asmar[1], J.F. Collet[1]
[1]Université catholique de Louvain, de Duve Institute, Brussels, Belgium

Backgrounds

Bacterial lipoproteins are a very diverse group of proteins characterized by the presence of an N-terminal lipid moiety that serves as a membrane anchor. Lipoproteins have a wide variety of crucial functions, ranging from envelope biogenesis to stress response. In Gram-negative bacteria, lipoproteins can be targeted to various destinations in the cell, including the periplasmic side of the cytoplasmic or outer membrane, the cell surface or the external milieu.

Objectives

One of the objectives of our lab is to understand how lipoproteins can reach the cell surface of Escherichia coli and other Gram-negative bacteria.

Methods

By focusing on the lipoprotein stress sensor RcsF, we dissected the molecular mechanism allowing this protein to become surface-exposed.

Conclusions

This allowed us to discover and specify the role played by beta-barrel proteins in this process.

FEMS7-1668
Physiology / Biochemistry / Molecular Microbiology

JANTHINOBACTERIUM SP. ROICE36 AS A PROMISING SOURCE OF NOVEL ANTIMICROBIAL COMPOUNDS

A. Baricz[1], C. Chiriac[1], E. Szekeres[1], C. Coman[1]
[1]Institute of Biological Research Cluj-Napoca- branch of NIRDBS Bucharest, Taxonomy and Ecology, Cluj-Napoca, Romania

Backgrounds

Since their discovery, antibiotics have changed the face of medicine, saving millions of lives from deadly infections. But, due to improper use of antibiotics, the phenomenon of antimicrobial resistance has been growing constantly. Natural products are an important source of novel antibiotics in modern medicine, and have made an unquestionable impact on global health so far. The *Janthinobacterium* sp. ROICE36 strain, isolated during the ROICE Romanian expeditions to Antarctica, is a promising candidate in the research for possible novel antimicrobials.

Objectives

To perform a biochemical characterization, to test several growth conditions and to sequence the genome in order to obtain an improved yield of bacterial biomass and to characterize the antimicrobial biosynthetic pathways.

Methods

Determine the carbohydrate utilization capability and the semiquantitation of enzymatic activities of the *Janthinobacterium* sp. ROICE36 strain. Characterize the strain's growth parameters on solid and in liquid media. Whole genome sequencing and description of the genome features.
Obtain the bacterial extract and test its antibacterial activity against multidrug resistant pathogens.

Conclusions

Species of the genus *Janthinobacterium* synthesize secondary metabolites that exhibit exceptional antibacterial, antifungal, antiviral and antiprotozoal activity. As the levels of resistance to some antibiotics in several important pathogens are growing worldwide, it is important to identify promising sources of novel antimicrobials with potential usefulness in biotechnological applications.

Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2016-1170, within PNCDI III.

FEMS7-1962
Physiology / Biochemistry / Molecular Microbiology

A STRESS-INDUCED REGULON REQUIRED FOR STAPHYLOCOCCAL INFECTION

M.D.M. Cordero Alba[1], *D. Lopez*[1]
[1]*Centro Nacional de Biotecnología CNB-CSIC, Biotecnología Microbiana, Madrid, Spain*

Backgrounds

A large number of bacterial infections are caused by opportunistic pathogens, like *Staphylococcus aureus,* which represents the most common source of infections in clinical setting due to the fact that it colonizes human skin and causes device contamination during surgical practice. *S. aureus* possesses different systems that can control cell-density that could be involved in infection, like the quorum-sensing (QS) system.

Objectives

In this report, we investigate the functionality of a new genetic regulon that is necessary for virulence in *Staphylococcus aureus* cells and is triggered by diverse types of cellular stresses.

Methods

We carry out a comprehensive number of omics approaches in combination to classical biochemical methods and *in vivo* animal experimentation.

Conclusions

We identify a new regulon that is triggered in specific conditions and is necessary for the progression of an infection. This regulon is not active during non-infective conditions. We will show the genes that constitute the regulon and will provide details about the genetic pathway that leads to the activation of the cascade *in vitro* and also *in vivo* using a murine infection model.

FEMS7-1248
Physiology / Biochemistry / Molecular Microbiology

SPATIAL TARGETING OF HETEROLOGOUS CLASS I PI3K IN SACCHAROMYCES CEREVISIAE AND DEVELOPMENT OF TOOLS FOR PHOSPHOINOSITIDE IN VIVO MONITORING

J.M. Coronas-Serna[1], T. Fernádez-Acero[1], V.J. Cid[1], M. Molina[1]
[1]Complutense University of Madrid - School of Pharmacy, Department of Microbiology II, Madrid, Spain

Backgrounds

Phosphoinositides play a key role in various signaling pathways. In mammals, *in situ* generation of PtdIns(3,4,5)P_3 by PI3K in plasma membrane (PM) regulates growth factor response through the Akt pathway. Heterologous expression of mammalian class I PI3K catalytic subunit p110α in the model organism *S. cerevisiae* leads to growth inhibition due to the conversion of essential PM PtdIns(4,5)P_2 into PtdIns(3,4,5)P_3, naturally absent in yeast.

Objectives

Our aim was to target PtdIns(4,5)P_2 removal to discrete PM domains by fusing p110α to either the Cdc10 septin or the eisosome component Pil1, as well as to develop a fluorescence double marker-based tool to study the localization of PI3K activity *in vivo* by simultaneous monitoring of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3.

Methods

Functional analyses on cells expressing chimaeras of p110α and distinctly localized yeast proteins. Construction of GFP/mCherry-based phosphoinositide-specific markers for fluorescence microscopy.

Conclusions

Expression of either Cdc10-p110α or Pil1-p110α fusions led to growth inhibition of yeast cells similar to that induced by N-myristoylated p110α, which is not targeted to specific PM domains. Both fusion proteins caused a delay in endosomal trafficking. Cdc10-p110α exclusively localized to the bud neck. A fusion of p110α to a Cdc10 mutant version, which failed to localize properly, did not inhibit growth, demonstrating that anchoring to the septin ring was required for toxicity. Single-cell monitoring of PM phosphoinositide species by the double marker plasmid developed revealed that, despite the spatially restricted activity of p110α, PtdIns(4,5)P_2 conversion to PtdIns(3,4,5)P_3 was observed along the whole PM.

FEMS7-3238
Physiology / Biochemistry / Molecular Microbiology

AS AFFECT THE ABSENCE OF NITROGEN OR CARBON PEP4, LAP4, PRC AND APE3 GENES OF CANDIDA GLABRATA

J.L. Cortez Sánchez[1], *J.A. Ibarra García*[2], *C.H. Hernández Rodríguez*[2], *M.D.L. Villa Tanaca*[1]
[1]*Instituto Politécnico Nacional, Microbiologia, Mexico, Mexico*
[2]*Instituto Politécnico Nacional, Microbiología, Mexico, Mexico*

Backgrounds

C. glabrata is an opportunistic pathogen, resistant to conventional antifungals such as azoles, is currently considered the second cause of disseminated candidiasis and mucosal candidiasis in humans. A great variety of virulence factors have been studied, but many are still to be described and control of *C. glabrata* infections. In other microorganisms it has been observed that the proteolytic system vacuolar is very important for its adaptation and survival. On the other hand, despite the relevance that *C. glabrata* has acquired, to date the *C. glabrata* vacuolar proteolytic system has not been studied in detail.

Objectives

This work seeks to understand the role of *PEP4*, *APE1, APE3, PRC* in absence of nitrogen, carbon or both and determine the conditions where induced expression of these genes and at the same time evaluate the specific activity of the proteins encoded by these genes.

Methods

A bioinformatic analysis was performed using the *S. cerevisiae* protein sequences to search for *C. glabrata*, the promoter sequences were analyzed using the genomatix server, the genes were analyzed by RT-qPCR and the specific activity of each of the proteins was determined.

Conclusions

Taken together our results showed that PrA, Ape1, Ape3 and CpY have varying activities and expression depending on whether nitrogen or carbon is added to the media and that these vacuolar proteases might have a role in the autophagy process.

FEMS7-1393
Physiology / Biochemistry / Molecular Microbiology

REGULATION OF THE SYNTHESIS AND SUBCELLULAR LOCALIZATION OF THE ESCHERICHIA COLI ZIPA DIVISION PROTEIN

L. Cueto[1], *A. Sanchez Gorostiaga*[1], *M. Vicente*[1]
[1]*Centro Nacional De Biotecnologia, Biotecnologia Microbiana, Madrid, Spain*

Backgrounds

The ZipA protein is essential for *Escherichia coli* division. It is a transmembrane protein solely present in gammaproteobacteria. Together with FtsA and FtsZ, they form the proto-ring, a protein complex required to initiate the assembly of the divisome. Transcription of the *ftsZ* and *ftsA* genes, located in the *E. coli dcw* (division and cell wall) cluster, is driven by a combination of gearbox (RpoS-dependent) and housekeeper (RpoD-dependent) promoters. However, *zipA* maps outside the *dcw* cluster and the signals controlling its expression need to be identified.

Objectives

Characterization of the *zipA* expression pattern in *E. coli* depending to the growth rate. Identification of the signals that regulate *zipA* expression. Determine if ZipA is specifically associated to particular membrane microdomains.

Methods

Use of reporter GFP-fusions to locate, sequences able to act as potential promoters and regulatory binding sequences in the 96 bp upstream region. Deletions in the *zipA* promoter regions to determine their effect on cell division. Measurements of the ZipA amount relative to cell mass in the *E. coli* MG1655 wild-type strain and an isogenic Δ*rpoS* strain at different growth rates and during different phases of population growth. The presence of ZipA in specific membrane microdomains was analyzed by Western blot using suitable membrane fractions.

Conclusions

In contrast to the growth rate-independency of the amounts of FtsA and FtsZ found per cell, the levels of ZipA in *E. coli* cells increase, as it occurs with other membrane proteins, accordingly to their growth rate. Finally, we could localize ZipA associated to specific cytoplasmic membrane microdomains.

FEMS7-2292
Physiology / Biochemistry / Molecular Microbiology

PHYSICAL INTERACTION BETWEEN THE MAPK SLT2 OF THE PKC1-MAPK PATHWAY AND GRX3/GRX4 GLUTAREDOXINS IS REQUIRED FOR THE OXIDATIVE STRESS RESPONSE IN BUDDING YEAST

M.A. de la Torre-Ruiz[1], N. Pujol-Carrion[1], A. Barbero Farran[1], M.A. Mechoud[1]
[1]Institute for Biomedical Research IRB LLEIDA, Basic Medical Sciences, Lleida, Spain

Backgrounds

Slt2 is the MAPK of the CWI pathway in budding yeast whereas Grx3/Grx4 are two redundant monothiol glutaredoxins with a role in iron homeostasis. Slt2/Grx3/Grx4 are functionally related in the oxidative response.

Objectives

To analyse at the molecular and biochemical level the physical interaction between the MAPK Slt2 and Grx3/Grx4 monothiol proteins.

To elucidate the biological function of Slt2/Grx3/Grx4 complex in the oxidative response.

Methods

See: Free Radic Biol Med. 2017. 103:107-120.

Conclusions

This study demonstrates that both monothiol glutaredoxins Grx3 and Grx4 physically interact with the MAPK Slt2 forming a complex involved in the cellular response to oxidative stress. The simultaneous absence of Grx3 and Grx4 provokes a serious impairment in cell viability, Slt2 activation and Rlm1 transcription in response to oxidative stress. Our results suggest that Slt2 form iron/sulphur bridged clusters with Grx3 and Grx4. For the assembly of this complex, cysteines of the active site of each Grx3/4 glutaredoxins, glutathione and specific cysteine residues from Slt2 provide the ligands. One of the ligands of Slt2 is required for its dimerisation upon oxidative treatment and iron repletion. These interactions are relevant for the oxidative response, given that mutants in the cysteine ligands identified in the complex show a severe impairment of both cell viability and Slt2 phosphorylation upon oxidative stress. Grx4 is the relevant glutaredoxin that regulates Slt2 phosphorylation under oxidative conditions precluding cell survival. Our studies contribute to extend the functions of both monothiol glutaredoxins to the regulation of a MAPK in the context of the oxidative stress response.

FEMS7-2026
Physiology / Biochemistry / Molecular Microbiology

IDENTIFICATION OF RCTB MUTATIONS THAT PROMOTE V. CHOLERAE ORI2 OVER-INITIATION

F. de Lemos Martins[1], *M.E. Val*[1], *D. Mazel*[1]
[1]*Institut Pasteur, Genomes and Genetics, Paris, France*

Backgrounds

Vibrio cholerae has two chromosomes, Chr1 (3 Mbp) and Chr2 (1 Mpb). Replication initiation of the two chromosomes is not synchronous but their termination is. Indeed, initiation of Chr2 replication is triggered when 2/3rd of Chr1 has been replicated. On Chr1, a binding site for the initiator of Chr2 (RctB) called *crtS* (Chr2 replication triggering Site) is crucial for the activation of Chr2 replication. We showed that the replication of *crtS* triggers Chr2 replication. This revealed a novel check-point control mechanism in bacteria in which one chromosome communicates with another chromosome to coordinate their replication. However the molecular basis behind this process is largely unknown.

Objectives

In this work we aimed to unravel potential factors involved in *V. cholerae* Chr2 replication regulation.

Methods

We performed an experimental evolution of *ΔcrtS* mutants. After growth for 200 generations these mutants acquired point mutations affecting exclusively *rctB*. By testing these mutations on a synthetic mini-chromosome based on *V. cholerae ori2* (mini-Chr2) we observed that they were able to stabilize it in *E. coli*, when it is normally lost without selection. By qPCR we found that all *rctB* mutated mini-Chr2 have a higher copy number suggesting that they over-initiate their replication.

Conclusions

Overall, our results show that the *rctB*-associated mutations are able to partially restore the defects caused by *crtS* deletion by altering Chr2 initiation regulation. This might suggest a direct interplay between *crtS* and RctB to coordinate chromosome replication in *V. cholerae*.

FEMS7-0305
Physiology / Biochemistry / Molecular Microbiology

AWAKENING DORMANT SECONDARY METABOLITE GENE CLUSTERS IN STREPTOMYCES

A. de Pedro[1], E.G. Barreales[1], Á. Rumbero[2], C.M. Vicente[1,3], T.D. Payero[1], J.F. Aparicio[1]
[1]Universidad de León, Biología Molecular, León, Spain
[2]Universidad Autónoma de Madrid, Química Orgánica, Madrid, Spain
[3]Present address: Université de Lorraine, Dynamique des Génomes et Adaptation Microbienne, Vandoeuvre-lès-Nancy, France

Backgrounds

Sequencing of *Streptomyces* genomes has unveiled that the secondary metabolite biosynthetic potential of these bacteria has been greatly underestimated. Most secondary metabolite gene clusters in these bacteria are silent (they are not expressed or they do little), and through their activation we can achieve the production of new molecules. Heterologous expression of the regulator PimM (an archetype of PAS-LuxR regulator) has been successfully used to trigger the production of several polyene antifungal macrolides in different producing strains, but also to boost the production of other compounds.

Objectives

Activation of silent secondary metabolite gene clusters for the discovery of new drugs.

Methods

A deregulated *pimM* gene where its original promoter has been replaced by a strong constitutive promoter was introduced in different *Streptomyces* strains, and the antibiotic and/or antifungal activity of the exconjugants was determined by bioassay. Subsequently, the novel activities identified were characterized by HPLC, and the responsible compounds purified and characterized by MS, and NMR structural studies.

Conclusions

Overexpression of *pimM* in different *Streptomyces* strains results in increased production of secondary metabolites of diverse nature such as polyketides: amphotericin in *S. nodosus*, filipin in *S. avermitilis* and *S. filipinensis,* or depsipeptide antimycins: in *S. albus* and *S. ambofaciens.* This makes PAS-LuxR regulators an excellent tool for the activation of silent gene clusters for secondary metabolites, and therefore for the discovery of new drugs.

FEMS7-0464
Physiology / Biochemistry / Molecular Microbiology

MULTIPLE GENE CLUSTER CONTROL BY A SINGLE PAS-LUXR TRANSCRIPTIONAL REGULATOR

C.M. Vicente[1,2], A. de Pedro[2], E.G. Barreales[2], T.D. Payero[2], J.F. Aparicio[2]
[1]Present address: Université de Lorraine, Dynamique des Génomes et Adaptation Microbienne, Vandoeuvre-lès-Nancy, France
[2]Universidad de León, Biología Molecular, León, Spain

Backgrounds

PAS-LuxR regulators are highly conserved proteins involved in the control of antifungal production by binding to operators located in given promoters of polyene biosynthetic genes. These regulators have been found to be encoded in all know biosynthetic gene clusters of polyene polyketides, and hence they have been considered pathway-specific modulators. PteF is the PAS-LuxR transcriptional activator of filipin biosynthesis in *Streptomyces avermitilis*.

Objectives

Identification of gene targets of regulatory control.

Methods

We have used the canonical operator of PimM, archetype of this class of regulators, to search for putative targets of orthologous protein PteF in the genome of *S. avermitilis*. Multiple DNA-binding sites for PimM (and therefore for PteF) were found in the genome. Several of these operators were selected and their binding to PimM DNA-binding domain was assessed by electrophoretic mobility shift assays. Additionally we carried out comparative transcriptome analyses in parental and mutant strains by microarrays. Transcriptomic results were validated by quantitative reverse transcription polymerase chain reaction, and by metabolite production studies.

Conclusions

Contrary to the established opinion, PAS-LuxR regulators control a plethora of different processes previously unforeseen, such as genetic information processing; DNA, energy, carbohydrate, and lipid metabolism; morphological differentiation; and transcriptional regulation; among others, but particularly secondary metabolite biosynthesis. Notably, fourteen secondary metabolite gene clusters out of 38 encoded by *S. avermitilis* genome, many of them encoding cryptic compounds, showed to be controlled by the regulator, suggesting a regulatory role for PteF wider than expected. This opens new possibilities for enhancement and awakening of metabolite production in *Streptomyces*.

FEMS7-1710
Physiology / Biochemistry / Molecular Microbiology

FUNCTIONAL ANALYSIS OF THE MYCOBACTERIUM SMEGMATIS MSMEG_3765 GENE, CODING A TETR-LIKE PROTEIN

F. Perrone[1], B. De Siena[1], L. Muscariello[1], S. Kendall[2], S. Waddell[3], M. Sacco[1]
[1]Università degli Studi della Campania Luigi Vanvitelli, Di.S.T.A.Bi.F., Caserta, Italy
[2]The Royal Veterinary College, Pathology and Pathogen Biology, London, United Kingdom
[3]University of Sussex, Medical School, Brighton, United Kingdom

Backgrounds

Mycobacterium tuberculosis has the ability to survive in the macrophage under acid- nitrosative stress. The expression profile of *M. tuberculosis* and *M. smegmatis* exposed to acid-nitrosative stress shows up-regulation of *M. smegmatis MSMEG_3765* and of its ortholog, *M. tuberculosis Rv1685c* (Cossu *et al.*, Microb. Pathog. 65:89, 2013). Both genes are annotated as TetR transcriptional regulators. This family of proteins regulates a wide range of cellular activities, including multidrug resistance, efflux pumps, virulence and pathogenicity. In the Mycobacteria genus they are the most abundant transcriptional regulator and the majority of them remains uncharacterized.

Objectives

The aim of this study was to identify the genes regulated by *MSMEG_3765* in order to further understand the role of this regulator in the stress response.

Methods

A deletion was made in *MSMEG_3765* by homologous recombination and changes in genome-wide expression levels as a result of the deletion were measured using microarray analysis. Local gene expression levels were also quantified by RTq-PCR and by measuring promoter activity using GFP reporters. The TetR regulator (MSMEG_3765) was expressed and purified and used to identify promoters bound.

Conclusions

MSMEG_3765 was found to regulate the adjacent operon (*MSMEG_3762-63-65).* The purified regulator was found to bind to the promoter region upstream of *MSMEG_3762-63-65*, but no TetR motif was predicted in this region. These results show that MSMEG_3765 regulates an efflux pump with an, as yet, undefined role in the stress response.

FEMS7-2880
Physiology / Biochemistry / Molecular Microbiology

BREAKING THE BOND BETWEEN HELICOBACTER PYLORI AND ITS HOST

A. Debraekeleer[1], F. Coppens[1], G. Castaldo[1], K. Moonens[1], H. Remaut[1]
[1]Vrije Universiteit Brussel, Structural and Molecular Microbiology, Brussel, Belgium

Backgrounds

Helicobacter pylori is a human stomach pathogen infecting over half the world's population. The infection is generally associated with asymptotic gastritis, but can progress to peptic ulcers and stomach cancers. To sustain infection, the bacteria maintain an intimate interaction with the stomach epithelial cells and overlaying mucus by using a set of structurally conserved autotransporter-like adhesins called Hops.

Objectives

A highly conserved C-terminal domain is believed to represent the Hop transmembrane domain, but lacks typical features seen in classical autotransporters. We here set out to investigate the Hop architecture and transport route to the outer membrane.

Methods

We use X-ray crystallography, directed mutagenesis and localization studies to delineate the Hop passenger and transmembrane domain. Recombinant expression in *E. coli* is used to determine the minimal Hop fragment that is targeted to the outer membrane as a stable b-barrel.

Conclusions

We found that Hops represent a novel family of autotransporter-like adhesins with a conserved discontinuous β-barrel, interrupted by the α-helical passenger domain in extracellular loop 1. The Hop architecture is incompatible with prevailing models for autotransporter insertion into and passenger transport across the outer membrane, leading us to further investigate the route and mechanism of Hop biogenesis as a means of targeting this family of primary *Helicobacter pylori* virulence factors.

FEMS7-0080
Physiology / Biochemistry / Molecular Microbiology

TUBERCULOUS LYMPHADENITIS IN ETHIOPIA PREDOMINANTLY CAUSED BY STRAINS BELONGING TO THE DELHI/CAS LINEAGE AND NEWLY IDENTIFIED ETHIOPIAN CLADES OF THE MYCOBACTERIUM TUBERCULOSIS COMPLEX

F.B. Degeneh[1]
[1]*Bahir Dar University, Medical Microbiology - immunology and parasitology, Bahir Dar, Ethiopia*

Backgrounds

Recently, newly defined clades of *Mycobacterium tuberculosis* complex (MTBC) strains, namely Ethiopia 1-3 and Ethiopia H37Rv-like strains, and other clades associated with pulmonary TB (PTB) were identified in Ethiopia.

Objectives

We investigated whether these new strain types exhibit an increased ability to cause TB lymphadenitis (TBLN) and raised the question, if particular MTBC strains derived from TBLN patients in northern Ethiopia are genetically adapted to their local hosts and/or to the TBLN.The study was conducted to extend our understanding of the diversity; phylogeny, and transmission dynamics of MTBC strains isolated from TBLN patients in Ethiopia

Methods

Genotyping of 196 MTBC strains was performed by spoligotyping and 24-loci mycobacterial interspersed repetitive unit-variable number of tandem repeats (MIRU-VNTR) typing. A statistical analysis was carried out to see possible associations between patient characteristics and phylogenetic MTBC strain classification.

Conclusions

In the studied area, strains obtained from lymph node samples are mainly belonging to the MTBC Dehli/CAS lineage. The lower cluster rate in this study as compared to previous studies on PTB patients might reflect the dead end characteristics of the disseminated form of the disease or may be associated to a higher rate of TBLN patients among TB reactivation events. We found no indication that strains of particular genotypes are specifically associated with TBLN. Nevertheless the identification and treatment of TB bacteria in TBLN patients is of great importance.

FEMS7-1572
Physiology / Biochemistry / Molecular Microbiology

REGULATION OF VIBRIO CHOLERAE COLONY MORPHOLOGY BY TEMPERATURE

T. del Peso-Santos[1], *J. Blake*[2], *V. Benes*[2], *F. Cava*[1]
[1]*Umeå University, Department of Molecular Biology, Umeå, Sweden*
[2]*European Molecular Biology Laboratory, Genomics Core Facility, Heidelberg, Germany*

Backgrounds

A common strategy for bacterial growth and survival is the formation of biofilms - bacterial communities found in surfaces and interfaces held together by an extracellular matrix composed by exopolysaccharides, proteins and extracellular DNA. The biofilm life style provides protection from the external environment, i.e. predators and physical and chemical stresses such as antibiotics, controls nutrient adsorption and enhances bacterial persistence.

Vibrio cholerae - an aquatic bacterium that is the causative agent of Cholera disease - forms biofilms during both the acquatic and intestinal phases of its life cycle. Among all the diverse environmental signals that regulate biofilm in *V. cholerae*, temperature is of major importance upon infection of a host and subsequent transmission, since this bacterium undergoes a temperature shift in its transition between the human host (37ºC) and the aquatic environment (lower temperatures).

Objectives

This work aims to unravel the molecular mechanism(s) behind temperature-dependent regulation of *V. cholerae*'s biofilm.

Methods

We have performed: (1) a global transcriptome analysis of *V. cholerae* at different temperatures that result in different colony morphology (rugose colonies = biofilm; smooth colonies= less or biofilm) by means of RNA seq; and (2) a massive transposon mutagenesis, in order to find crucial activities important for this temperature-dependent regulation.

Conclusions

The identified activities that are involved in temperature-mediated regulation of *V. cholerae*'s biofilm are presented here.

FEMS7-0393
Physiology / Biochemistry / Molecular Microbiology

OBGE* CAUSES CELL DEATH BY INTERFERING WITH CELL DIVISION

L. Dewachter[1], *N. Verstraeten*[1], *M. Fauvart*[1,2], *J. Michiels*[1]
[1]*KU Leuven, Centre of Microbial and Plant Genetics, Leuven, Belgium*
[2]*imec, Smart Systems and Emerging Technologies Unit, Leuven, Belgium*

Backgrounds

Although intensely studied, cell division remains a universal core process that is incompletely understood. Even in well-studied organisms, such as *Escherichia coli*, many factors involved in cell division remain to be identified or are not fully characterized.

Objectives

We here demonstrate a role for the widely conserved GTPase, ObgE, in the regulation of cell division in *E. coli*. We do so by showing that a mutant form of this protein, ObgE*, interferes with division. Insights gleaned from characterizing the effect of ObgE* can reveal more about the role of wild-type ObgE in cell division.

Methods

Morphological changes upon ObgE* expression indicate that ObgE* can inhibit the separation of newly-formed daughter cells and cause the formation of cell chains. Lysis then occurs through rupture of membrane blebs that form at division sites. Additionally, in the absence of lysis, ObgE* can block cell division before constriction has started.

Conclusions

Cell death caused by ObgE* occurs either through an irreversible block in cell division or through inhibition of cell separation. Since a defect in division underlies both pathways, these results strongly implicate ObgE in the regulation of cell division in *E. coli*. Because Obg proteins are widely conserved and essential for bacterial viability, our findings might be applicable to other organisms as well.

FEMS7-2115
Physiology / Biochemistry / Molecular Microbiology

NADPH REGENERATION AND REDOX BALANCE SYSTEMS IN OENOCOCCUS OENI ETHANOL-STRESSED CELLS

K. Di Tomaso[1,2], M.G. Bonomo[1], L. Calabrone[1], G. Salzano[1]
[1]Università degli Studi della Basilicata, Sciences, Potenza, Italy
[2]Università degli Studi della Basilicata, Ph.D school in Applied Biology and Environmental Safeguard, Potenza, Italy

Backgrounds

Various physical/chemical factors, such as ethanol, pH and temperature, are known to affect the growth and bacterial survival of *Oenococcus (O.) oeni* that faces the hostile environment of wine by activation of several mechanisms.Ethanol tolerance appeared to be a crucial feature for the activity of *O. oeni* cells in wine because ethanol acts as disordering agent of the *O. oeni* cell membrane and negatively affects metabolic activity.

Objectives

A transcriptomic analysis of *O. oeni* ethanol stressed cells by NGS approach allows to investigate variations in transcripts involved in energy metabolism pathways and changes in *O. oeni* metabolic profile induced by ethanol stress.

Methods

In this study RNA-seq was used to assemble *O. oeni* S12 transcriptome and detect genes differentially expressed in ethanol stress conditions.
Results showed changes in pentose phosphate and ethanol forming pathway, i.e. the overexpression of 6-phosphogluconate dehydrogenase and short chain alcohol dehydrogenase genes, due to the competition for NAD(P)H, in stressed cells. Two systems for cofactor regeneration were revealed: NADPH oxidoreductase activity, overexpressed in stressed cells, and an alternative use of pyruvate, shifted in diacetyl, acetoin and 2,3-butanediol, confirmed by the upregulation of acetoin reductase transcript.

Conclusions

NGS approach revealed ethanol stress triggers deep changes in *O. oeni* transcriptome to ensure cell viability, resulting a valid tool for studying stress responsive mechanisms and to improve knowledge on *O. oeni* adaptive response to ethanol stress.

FEMS7-3075
Physiology / Biochemistry / Molecular Microbiology

COMPARATIVE METABOLOMICS ANALYSIS BETWEEN MYCOBACTERIUM TUBERCULOSIS AND A TUBERCULOSIS VACCINE CANDIDATE MTBVAC

C. Diaz Navarro[1], J. Pérez-del Palacio[1], P. Mena[1], A. Sánchez Pozo[2], O. Genilloud[3], C. Martín[4,5,6], F. Vicente[1], J. Gonzalo-Asensio[4,6,7]
[1]Fundación MEDINA, Screening and target validation, Armilla, Spain
[2]Facultad de Farmacia Universidad de Granada, Departamento de Bioquímica y Biología Molecular II, Granada, Spain
[3]Fundación MEDINA, Microbiology, Armilla, Spain
[4]Facultad de Medicina Universidad de Zaragoza,
Departamento de Microbiología y Medicina Preventiva, Zaragoza, Spain
[5]Hospital Universitario Miguel Servet, Servicio de Microbiología, Zaragoza, Spain
[6]Instituto de Salud Carlos III, CIBER Enfermedades respiratorias, Zaragoza, Spain
[7]Instituto de Biocomputación y Física de Sistemas complejos,
Instituto de Biocomputación y Física de Sistemas complejos, Zaragoza, Spain

Backgrounds

MTBVAC is a live attenuated *M. tuberculosis* vaccine based on two independent stable deletion mutations without antibiotic resistance markers in the virulence genes *phoP* and *fadD26*. MTBVAC is being tested in clinical trials. At present there are no studies to characterize metabolic expression in the attenuated strain

Objectives

Find the differences in expression of metabolites between strains and the involvement of the *phoP* gene in the differences in metabolite expression, and characterize the associated changes in pathogenicity and activation of the immune system.

Methods

Five replicas of *Mycobacterium tuberculosis* and five replicas of MTBVAC were cultivated and their supernatants were analyzed by liquid chromatography coupled to high resolution mass spectrometry with four different conditions. Reverse-phase and HILIC chromatographic modes were applied to deal with highly polar as well as hydrophobic metabolites required for untargeted metabolomics and positive and negative ionization

Conclusions

Using a metabolomics approach, we identified specific metabolites that may be used to characterize the genomic differences. We found about 200 differential metabolites significantly in the four analyses. Within the molecules that have been identified, there are different components involved in the formation of membranes, lipids, mainly phosphatidylinositol and molecules directly involved in the metabolism, such as nucleotide, glutamine or glutamate. Through the tentatively identified metabolites, a relationship can be established that helps elucidate mechanistic pathways for a better understanding about pathogenicity and potentiation of the immune system.

FEMS7-1175
Physiology / Biochemistry / Molecular Microbiology

FUNCTIONAL STUDY OF THE ROLE OF BACTERIAL CYCLOPHILIN PPIB IN CELL DIVISION

A. Skagia[1], C. Zografou[1], A. Venieraki[1], C. Fasseas[1], P. Katinakis[1], M. Dimou[1]
[1]Agricultural University of Athens, Faculty of Crop Science, Athens, Greece

Backgrounds

E. coli PpiB belongs to the superfamily of peptidyl prolyl *cis*/*trans* isomerases (PPIase, EC: 5.2.1.8), which are conserved among organisms and are implicated in many biological processes in addition to protein folding. Here we show that the Δ*ppiB* deletion strain and the PpiB overexpression wild type strain are both characterized by defects in cell division involving milder or severe cell filamentation, respectively.

Objectives

We aim to identify whether PpiB interfering with cell division occurs via one or more components of the divisome machinery and/or via additional protein interactions not directly involved in the division process. Furthermore, we aim to identify the essential PpiB structural features for the inhibitory effect of PpiB overexpression in cell division.

Methods

We express various divisome components or putative PpiB prey proteins in Δ*ppiB* cells and check for phenotype restoration. We examine the protein interaction of PpiB with FtsZ under native or denaturing conditions and we further assess their association using enzymatic and localization studies. We express various PpiB mutants in wild type cells and observe the effect on cell morphology.

Conclusions

PpiB is involved in bacterial cell division in a way that employs its prolyl isomerase activity along with structural features apart from the catalytic site as well as its association with FtsZ. However, PpiB seems to also modulate the function of additional proteins not previously related to cell division.

FEMS7-0250
Physiology / Biochemistry / Molecular Microbiology

SCREENING FOR COMPOUNDS WHICH SPECIFICALLY INHIBIT THE YEAST MULTIDRUG TRANSPORTER PDR5

K. Döhl[1], S.H.J. Smits[1], L. Schmitt[1]
[1]Heinrich-Heine-University- Duesseldorf, Institute of Biochemistry, Duesseldorf, Germany

Backgrounds

The ABC transporter Pdr5 is part of the pleiotropic drug resistance (PDR) network of the yeast *Saccharomyces cerevisiae* and plays a major role in drug resistance against a large number of structurally unrelated compounds. Pdr5 is a functional homologue of Cdr1 from the clinical relevant fungi *Candida albicans*.

Objectives

Consequently, the development of new inhibitors against these multidrug exporter proteins plays an important role in our fight against resistance fungi.

Methods

With a two-fold *in vitro* and *in vivo* strategy, we determined the inhibitory effect of unknown compounds to the yeast protein Pdr5. We were able to identify several substances, which inhibit the ABC transporter *in vitro*. To analyze the inhibitory effect *in vitro* we isolated highly enriched plasma membranes of a Pdr5 overexpressing yeast strain. We performed a high throughput assay monitoring the transport process of Pdr5 in real time. Several substances were identified to inhibit Pdr5 specifically. The oligomycin-sensitive Pdr5 ATPase was used as a second measurement to confirm and further characterize the inhibitory effect of these substances.
In vivo, a liquid drug assay was used to demonstrate that these substances are able to inhibit the transport as well. Therefore, we incubated Pdr5 wild type and an ATPase deficient mutant with different concentrations of the previously identified substances.

Conclusions

So far, none of the compounds that were identified *in vitro* inhibit Pdr5 *in vivo*. A reason could be that the substances are not able to cross the membrane efficiently or that much higher concentrations of the compounds are necessary.

FEMS7-1649
Physiology / Biochemistry / Molecular Microbiology

MYCOPLASMA CHROMOSOMAL TRANSFER: A NEW GATEWAY FOR EVOLUTION AND INNOVATION IN MINIMAL BACTERIA

E. Dordet-Frisoni[1], E. Sagne[2], E. Baranowski[1], X. Nouvel[2], C. Citti[1]
[1]INRA, IHAP- Université de Toulouse- INRA- ENVT- Toulouse- France, Toulouse, France
[2]ENVT, IHAP- Université de Toulouse- INRA- ENVT- Toulouse- France, Toulouse, France

Backgrounds

Horizontal gene transfer (HGT) is a major driving force of bacterial evolution and innovation. In mycoplasmas, this phenomenon was long thought to be marginal because of these bacteria having very few recombination systems and genetic mobile element. Mycoplasmas represent the simplest, self-replicating bacteria due to a minute-size genome whose evolution is driven by genetic losses. Our data recently challenged this dogma by showing the occurrence of significant HGT between ruminant mycoplasmas and, in some strains, of putative integrative conjugative elements (ICE). We demonstrated that mycoplasma chromosomal transfers (MCT) occur via conjugation using a new mechanism that does not conform to classic oriT-based conjugation models. While MTC and classical ICE transfer take place in the same population, MCT is independent from ICE movement.

Objectives

The purpose of this study is to understand the mechanisms underlying MCT

Methods

NGS sequence data from entire mating progenies and from several, individual transconjugants were compared.

Conclusions

Results showed that a single Mycoplasma recipient chromosome can acquire several segments of donor DNA. Transferred segments range from ~1,500 bp to ~80,000 bp in length and are exchanged with their recipient orthologs all around the genome. Transconjugant genomes contained up to 10% of donor-derived chromosome, distributed over 7 segments. MCT involves a recombination process, with micro-homologous regions as short as 12 nt flacking the transferred segment.
The discovery of MCT radically change our current views concerning the evolution of mycoplasmas, with particularly far-reaching implications given that over 100 species are human or animal pathogens.

FEMS7-0846
Physiology / Biochemistry / Molecular Microbiology

PROTEOMIC ANALYSES OF STREPTOCOCCUS SUIS DURING EXPOSURE TO HUMAN MACROPHAGES

K. Duangsonk[1], P. Prangsuwan[1], H. Thananchai[1], S. Roytrakul[2]
[1]Faculty of Medicine- Chiang Mai University, Department of Microbiology, Maung Chiang Mai, Thailand
[2]National Center for Genetic Engineering and Biotechnology, Proteomics Research Laboratory, Pathum Thani, Thailand

Backgrounds

Streptococcus suis is a zoonotic bacterium that can cause septicemia, meningitis and permanent hearing loss in human. Previous studies have shown that *S. suis* is able to resist phagocytosis, however, a small number of *S. suis* can enter and survive inside phagocytes. In an effort to understand the molecular mechanisms underlying *S. suis* adaptation, we investigated *S. suis* proteomes during exposure to intracellular condition of cultured human macrophages.

Objectives

This study was aimed to investigate the proteomic profiles of *S. suis* serotype 2 and 14 strains during exposure to intracellular condition cultured human macrophages U 937.

Methods

Cultured human macrophage U937 cells were infected with *S. suis* serotype 2 and 14 strains isolated from healthy pigs and human patients in northern Thailand. The protein patterns of the intracellular bacteria were analyzed by the one-dimensional electrophoresis combined with identification of proteins by the liquid chromatography mass spectrometry (LC-MS/MS).

Conclusions

The results indicated that all *S. suis* strains differentially expressed 118 proteins during exposure to intracellular condition of cultured human macrophages U937. Most of them were translation-associated proteins such as translation initiation factor IF-2, 30S ribosomal protein S7, 50S ribosomal protein L33 and Elongation factor G. These results indicated that all *S. suis* strains differentially adapted themselves to stress-conditions in human macrophages. These findings may lead to better understanding about the adaptation of this bacterium inside the harsh environment of host cells and the pathogenesis of *S. suis*.

FEMS7-1818
Physiology / Biochemistry / Molecular Microbiology

A PUTATIVE ADP-RIBOSYLTRANSFERASE TOXIN ASSOCIATED WITH AN RHS SYSTEM INHIBITS CELL DIVISION IN E. COLI

B. Dumont[1]
[1]*Free University of Brussels ULB, Laboratory of Bacterial Genetics and Physiology, Gosselies, Belgium*

Backgrounds

Contact-dependent growth inhibition (CDI) systems and rearrangement hotspots (*rhs*) are coding for long conserved proteins containing extremely variable C-terminal toxin domains (CdiA-CT/Rhs-CT). In addition, they also encode small cognate immunity proteins that antagonize the toxic domain activity (CdiI/RhsI). These systems were recently shown to play an important role in inter-bacterial competition as well as biofilm formation and are likely to possess still unknown functions.

Objectives

We investigated an Rhs-CT toxic domain from *X. bovienii* predicted to be an ADP-ribosyltransferase and analyzed its effect and mechanism of action in *E. coli*.

Methods

Validation of the toxic domain and its cognate immunity protein was performed by expression of the proteins in *E. coli*. Fluorescence microscopy technics were used to monitor morphological effects after toxin induction using DAPI staining and an FtsZ-GFP fusion. Inactive toxin mutants were constructed. We are currently setting up *in vitro* experiments to purify toxin-target complexes to identify the target.

Conclusions

We experimentally validated the functionality of the Rhs-CT/RhsI toxin-immunity pair in *E. coli*. Upon overexpression, the Rhs-CT toxin induces a strong filamentation phenotype while DNA replication and nucleoid segregation appear to be unaffected. Furthermore, filamentation appears to be independent of the SOS system. Using an FtsZ-GFP fusion, we observed that the Z-ring is destabilized upon toxin overexpression. Altogether, these data indicate that the Rhs-CT toxin directly inhibits cell division. The direct target is under investigation.

FEMS7-2853
Physiology / Biochemistry / Molecular Microbiology

THE ROLE OF DIVALENT METAL ION IN THE SELF-ASSEMBLY OF TET-AMINOPEPTIDASES

R. Dutoit[1], N. Brandt[2], V. Durisotti[2], C. Bauvois[2], D. Van Elder[3], L. Droogmans[3]
[1]Université Libre de Bruxelles - IRMW, Molecular biology Dept, Brussels, Belgium
[2]IRMW, NA, Brussels, Belgium
[3]Université Libre de Bruxelles, Molecular Biology Dept, Charleroi, Belgium

Backgrounds

The TET-aminopeptidases are dinuclear metallopeptidases requiring two divalent metal ions. These enzymes are able to self-assemble into a tetrahedron-shaped structure with 12 subunits. Their quaternary structure consists in the association of six dimers to form the functionally active dodecamer. In *Pyrococcus horikoshii*, both dimers and dodecamers co-exist *in vivo*, suggesting the self-assembly is regulated. However, their physiological function is unknown and the self-assembly is ill described due to the lack of structure of dimers. The divalent metal ions could also play an important role in the self-assembly.

Objectives

To unravel the role of the metal ions in the self-assembly, we have studied TmPep1050, a TET-aminopeptidase from *Thermotoga maritima*. This enzyme could be a model to understand the balance between dimers and dodecamers.

Methods

TmPep1050 was studied through a biochemical and structural approach. Its structure was solved by X-ray crystallography and its substrate specificity was determined with chromogenic substrates. To understand the role of metal ions in the oligomerization, several residues of the catalytic sites were mutated.

Conclusions

TmPep1050 self-assembles into a tetrahedron-shaped structure. This enzyme is cobalt-activated with broad substrate specificity and is highly thermostable at 75°C. The loss of cobalt ions led to a dramatic decrease of thermostability and the disassembly of dodecamers into dimers. The structure of the dimer showed the catalytic site is strongly impaired with a collateral impact on the dodecamerization interface. This phenomenon was observed in mutants unable to self-assemble. The self-assembly seems to depend on a correctly folded active site triggered by the presence of metal ions.

FEMS7-1154
Physiology / Biochemistry / Molecular Microbiology

ORAL MICROBIOTA DEVELOPMENT DURING CHILDHOOD IN RELATION TO DELIVERY MODE, BREASTFEEDING PRACTICES AND CARIES AT 9 YEARS OF AGE

M. Dzidic[1], M. Stensson[2], M.C. Collado[3], A. Artacho[1], T. Abrahamsson[4], M. Jenmalm[5], A. Mira[1]
[1]FISABIO Public Health, Genomics and Healthy, Valencia, Spain
[2]Jönköping University, Centre for Oral Health- School of Health and Welfare, Jönköping, Sweden
[3]Institute of Agrochemistry and Food Technology- Spanish National Research Council IATA-CSIC, Department of Biotechnology- Unit of Lactic Acid Bacteria and Probiotics, Valencia, Spain
[4]Linköping University, Department of Clinical and Experimental Medicine- Division of Paediatrics, Linköping, Sweden
[5]Linköping University,
Department of Clinical and Experimental Medicine- Unit of Autoimmunity and Immune Regulation, Linköping, Sweden

Backgrounds

The development of the infant microbiome is instrumental for human health. Although much is known about gut microbial colonization, information about early microbiome development in the oral cavity is scarce.

Objectives

We aimed to determine the acquisition and maturation of oral microbiota in saliva samples collected at 3,6,12, 24 months and 7 years of age in 90 children.

Methods

This was performed by using 16S-rRDNA high-throughput *Illumina* sequencing and other molecular techniques.

Conclusions

Bacterial diversity and richness increased steadily throughout childhood. Bacterial composition patterns changed through time and started with colonizers associated with the initial breastfeeding period, including *Lactobacillus* and *Veillonella,* while bacterial genera such as *Neisseria* and *Gemella* settled after two years of age. Infants born by C-section had initially skewed bacterial composition, compared to vaginally-delivered babies, although this recovered with age. Presence of caries at 9 years of age was associated with differences in colonization patterns at 7 years of age, with similar tendencies observed already at 2 years. No specific bacterial genera could be associated with increased risk of developing tooth decay, however. Partial breastfeeding until 12 months of age was associated with a different bacterial composition and the altered colonization patterns was enlarged with age.

This is the first longitudinal study where the salivary microbial colonization has been analysed by high-throughput sequencing. The data suggest that an altered colonization pattern during the first years of life may have long-term consequences for oral microbiome development, and the potential impact for the child's oral and systemic health should be further studied.

FEMS7-1300
Physiology / Biochemistry / Molecular Microbiology

REWINDING THE TAPE OF EVOLUTIONARY INNOVATION OF INTEGRON INTEGRASES

J.A. Escudero[1], A. Nivina[2], H. Kemble[3], A. Babic[2], C. Loot[2], O. Tenaillon[3], D. Mazel[2]
[1]Universidad Complutense de Madrid, Sanidad Animal, Madrid, Spain
[2]Institut Pasteur, Genomes and Genetics, Paris, France
[3]Universités Paris Diderot et Paris Nord Sorbonne Paris Cité-,
Infection- Antimicrobiens- Modélisation- Evolution IAMEpar, Paris, France

Backgrounds

Integrons are genetic platforms that allow bacteria to evolve rapidly through the acquisition of new genes encoded in cassettes and their differential expression. They are key players in the rise of multidrug resistance. Recombination in integrons is governed by integrases, remarkable proteins from an evolutionary perspective, as they represent molecular examples of evolutionary innovation. Modern integrases innovated to recombine single stranded (ss-)DNA, while retaining low levels of their ancestral activity, the recombination of exclusively double stranded (ds-)DNA molecules.

Objectives

We sought to obtain integrases that are hyperactive on ds-DNA, resembling ancestral integrases at the moment of evolutionary divergence.

Methods

We have carried directed evolution experiments using the *intI1* gene and two synonymous sequences to explore a broader protein sequence space. Among the three alleles, we found 8 independent mutations that enhanced integrase activity. Together they increased recombination rates 100-fold. All mutations were inserted into the three alleles and were re-evolved, yielding five new beneficial mutations. Negative epistasis was a burden in the process. We designed an epistasis purification system building a library of all possible combinations of the 13 mutations and subjecting it to our selective cycles. We now have alleles showing a 10.000 fold increase in activity with only 8 mutations.

Mutations revealed the structural constraints for ds-DNA recombination. They confirmed the pivotal role of integrase-specific I2 domain in innovation towards ss-DNA recognition. Hyperactivity on ds-DNA came with an asymmetric trade-off on ss-DNA.

Conclusions

We have successfully rewound the tape of evolutionary innovation in integrases by exploring a broader evolutionary landscape.

FEMS7-0561
Physiology / Biochemistry / Molecular Microbiology

THERAPEUTIC EFFECT OF RESVERATROL ON RESPIRATORY INFECTION BY NONTYPABLE HAEMOPHILUS INFLUENZAE

B. Euba[1,2], N. López-López[1], I. Rodríguez-Arce[1], A. Fernández-Calvet[1], N. Caturla[3], R. Díez-Martínez[4], J. Garmendia[1,2]
[1]Instituto de Agrobiotecnología- CSIC-Universidad Pública Navarra-Gobierno Navarra, Sanidad Animal, Mutilva, Spain
[2]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias, CIBERES, Madrid, Spain
[3]Monteloeder- Elche Parque Empresarial- Elche, Monteloeder, Alicante, Spain
[4]The Zebrafish Lab- Centro Europeo de Empresas e Innovación de Navarra, The Zebrafish Lab, Noáin, Spain

Backgrounds

Therapies that are safe, effective and not vulnerable to developing resistance are highly desirable to counteract bacterial infections. The respiratory pathogen nontypeable *Haemophilus influenzae* (NTHi) is an important cause of acute exacerbation of chronic obstructive pulmonary disease (COPD) that requires novel efficient treatments. A previous screening for host cell genes differentially expressed upon NTHi infection identified a repertoire of candidates to be targeted by host-directed therapeutics (1), including *sirtuin 1*, which encodes a NAD-dependent deacetylase, protective against emphysema by acting as a negative regulator of matrix metalloproteinase-9 (MMP9). COPD relates to an inappropriate MMP9 elevation, and SIRT1 activation may be a useful therapeutic approach. SIRT1 is activated by the plant polyphenol immunomodulator resveratrol.

Objectives

Evaluation of resveratrol therapeutic potential against respiratory infection by NTHi.

Methods

Resveratrol antimicrobial effect was determined for a collection of NTHi COPD clinical isolates by susceptibility assay. Resveratrol modulatory effect on the NTHi-host cell interplay was analyzed in terms of bacterial location upon infection, anti-inflammatory and anti-oxidative effects, by using cultured human airway epithelial cells. Resveratrol therapeutic potential was tested *in vivo*, by using both zebrafish septicemia and mouse respiratory NTHi infection model systems.

Conclusions

Resveratrol decreases NTHi viability in a dose-dependent manner through a bacteriostatic effect, and reduces airway epithelial inflammatory response upon NTHi infection. Resveratrol shows a significant bacterial clearing effect *in vivo*, without signs of host toxicity. These results highlight the antimicrobial potential of resveratrol against NTHi respiratory infection.

References

(1) Euba B *et al*. (2015) Antimicrob Agents Chemother 59(12):7581-92.

FEMS7-0984
Physiology / Biochemistry / Molecular Microbiology

OUTER MEMBRANE VESICLES OF THE PROBIOTIC ESCHERICHIA COLI NISSLE 1917 ACTIVATE THE NOD-1 SIGNALLING PATHWAY IN INTESTINAL EPITHELIAL CELLS

M.A. Canas Pacheco[1], M.J. Fabrega[1], C.S. Alvarez[1], R. Gimenez[1], J. Badia[1], L. Baldoma[1]
[1]University of Barcelona, Biochemistry and Animal Physiology, Barcelona, Spain

Backgrounds

To distinguish between pathogen and commensal bacteria intestinal epithelial cells are equipped with a range of pattern recognition receptors (PPRs), which specifically detect bacterial molecules and trigger the innate immune response. Among PPRs, the intracellular receptors NOD-1 and NOD-2 recognize intracellular peptidoglycan (PG) delivered by internalized pathogens or outer membrane vesicles (OMVs).

Objectives

The main objective of this work is to study whether OMVs released by the probiotic *Escherichia coli* Nissle 1917 (EcN) activate NOD1 and/or NOD-2 signalling pathways.

Methods

By using confocal fluorescence microscopy we have shown that EcN OMVs are internalized in Caco-2 cells and interact with both NOD1 and the endosomal protein EEA-1. To assess OMV-specific immune responses through NOD-1 activation we analysed expression of IL-6 and IL-8 in Caco-2 cells transfected with siRNA to knockdown NOD-1 levels (NOD1-KD). RT-PCR analysis revealed that NOD1-KD cells stimulated with EcN OMVs showed reduced significantly mRNA expression of IL-6 and IL-8, compared with control cells. Consistently, secreted levels of both cytokines measured by ELISA were lower in NOD1-KD cells, although values were only statistically significant for IL-6. No differences between control and NOD2-KD Caco-2 cells were observed. The study was completed by measuring degradation of the inhibitor IKB-alpha by Western blot to evaluate activation of NF-KB.

Conclusions

Overall, this study showed for the first time interaction of EcN OMVs with NOD1 and the activation of the innate immunity that results in a pro-inflammatory response.

FEMS7-1738
Physiology / Biochemistry / Molecular Microbiology

DISSECTING THE ROLE OF THE SMALL RNA ERSA IN PSEUDOMONAS AERUGINOSA MOTILITY AND BIOFILM REGULATION

M. Falcone[1], S. Ferrara[1], E. Rossi[2], H.K. Johansen[2], S. Molin[3], G. Bertoni[1]
[1]University of Milan, Biosciences, Milan, Italy
[2]Rigshospitalet, Clinical Microbiology, Copenhagen, Denmark
[3]Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Kgs. Lyngby, Denmark

Backgrounds

ErsA is a novel *Pseudomonas aeruginosa* small RNA responsive to infection-relevant host stimuli. Its transcription is under the control of the alternative sigma factor σ^{22} (AlgT/U), implicated in bacterial virulence.
In strain PAO1, ErsA exerts a direct negative post-transcriptional regulation in an incoherent feed-forward loop with σ^{22} on the bi-functional enzyme AlgC, which is involved in the biosynthesis of sugar precursors for alginate and polysaccharide exoproducts.

Objectives

We aim at the characterization of novel target genes regulated by ErsA to expand the knowledge about the ErsA-based regulatory network.

Methods

We employed different approaches: i) bioinformatics analysis, ii) *in vitro* electrophoretic mobility shift assays, iii) *in vivo* translational fusions based on the *gfp* reporter gene, iv) RNA-seq, and v) biofilm and motility assays on mutant strains.

Conclusions

Our results comprise:

i) *In silico* analyses identified new putative target genes mainly involved in biofilm formation and exopolysaccharides production.

ii) Among these targets, we specifically validated the interaction between ErsA and *amrZ* mRNA, both *in vitro* and *in vivo*

iii) The RNA-seq analysis showed that some of the genes whose expression is positively controlled by ErsA are important for biofilm development (i.e. genes belonging to the *pel* operon, *algD*).

iv) In line with the other results, the ErsA deletion mutant shows a hyper-motile phenotype compared to the wildtype and develops a thin and flat biofilm.

Overall, our results suggest that the small RNA ErsA might represent a relevant post-transcriptional regulator in biofilm development at different levels, likely interacting with different mRNA targets.

FEMS7-1871
Physiology / Biochemistry / Molecular Microbiology

ENGINEERING OF NOVEL RECOMBINANT ESCHERICHIA COLI STRAIN – A SOURCE OF BETA-GALACTOSIDASE

U. Falkouskaya[1], *A. Kastsianevich*[1], *L. Sapunova*[2]
[1]*Institute of Microbiology- National Academy of Sciences- Belarus, Laboratory of protein, Minsk, Belarus*
[2]*Institute of Microbiology- National Academy of Sciences- Belarus, Laboratory of enzymes, Minsk, Belarus*

Backgrounds

Beta-galactosidase is glycosidase which hydrolyzes beta-glycosidic bond formed between beta-D-galactosides, including disaccharide lactose, with glucose and galactose as the end products of the reaction. This enzyme is important for organisms as a key provider in the production of energy and a source of carbon released through break down of lactose to galactose and glucose. It is also vital for lactose intolerant community as it is responsible for making lactose-free milk and other dairy products.

Objectives

The study was aimed at engineering of novel recombinant *E. coli* strain– a source of beta-galactosidase.

Methods

PCR, POE-PCR, screening, electrophoretic analysis, chromatography.

Conclusions

A novel recombinant *E. coli* strain producing beta-galactosidase of *Arthrobacter sulfonivorans* was constructed by genetic engineering methods.

The specific primers for the gene *B-gal* were designed. This gene was amplified by PCR technique. The vector pET42a(+) was linearized with primers in such a way that a sequence of nucleotides that provides for presence of an additional octa-histidine oligopeptide at the target protein C-terminus was added into target gene 3'-terminus. The method of prolonged overlap extension PCR was applied for cloning gene encoding beta-galactosidase. The cells of strain *E. coli* BL21 were transformed by POE-PCR products. Six plasmid–containing colonies were selected by PCR-screening. After induction with 0.1 mM IPTG the target protein was purified by metal affinity chromatography. Biochemical properties of purified beta-galactosidase will be estimated at the next stage of research.

FEMS7-0694
Physiology / Biochemistry / Molecular Microbiology

MOLECULAR BASIS OF MEMBRANE POTENTIAL-GENERATING SYSTEM IN STAPHYLOCCOUS AUREUS

S.H. Fan[1], S. Reichert[1], F. Goetz[1]
[1]Interfaculty Institute for Microbiology and Infection Medicine Tübingen IMIT, Microbial Genetics, Tuebingen, Germany

Backgrounds

In many aerobic or facultative microorganisms, the major electron donor is $NADH+H^+$ produced during glycolysis and TCA cycle. In *Escherichia coli,* this is accomplished by NADH:quinone oxidoreductase (Ndh1). *Staphylococcus aureus* does not possess such complex, but a non-electrogenic type 2 (Ndh2) protein that oxidizes $NADH+H^+$. In addition, a protein called MpsA (*mps* for membrane potential-generating system) was identified in *S. aureus* showing sequence similarity to the proton-translocating subunit NuoL of complex 1 in *E. coli*. *mpsA* is the first part of an operon comprising three genes: *mpsA, mpsB and mpsC*. MpsB and MpsC show no significant sequence homologies to proteins with known function. However, MpsB carries a conserved metal binding motif similar to a domain that is found in carbonic anhydrases (CA).

Objectives

CA catalyzes the interconversion of $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. MpsB in *S. aureus* might play a role in CO_2 transport and respiration. Therefore, this study aims to investigate the involvement of MpsB in respiration, especially in CO_2 metabolism.

Methods

Deletion mutant of the *mpsB* and its complementation vector were constructed. Preliminary experiments were performed to characterize its growth on solid and liquid medium in aerobic and high (5%) CO_2.

Conclusions

Under normal conditions the growth of Δ*mpsB* was severely affected; however, in the presence CO_2 it was almost like the wild type. The pH profile of the mutant was also altered compared to the wild type. With regard to these observations, MpsB in *S. aureus* is postulated to be an integral part in CO_2 metabolism and energy conservation.

FEMS7-1273
Physiology / Biochemistry / Molecular Microbiology

HORIZONTAL CHROMOSOMAL TRANSFER: AN ACCELERATOR FOR THE EMERGENCE OF ANTIBIOTIC RESISTANCE IN MYCOPLASMAS ?

M. Faucher[1], L.X. Nouvel[1], E. Dordet-Frisoni[1], M. Marenda[2], F. Tardy[3], C. Citti[1]
[1]ENVT- INRA, UMR IHAP, TOULOUSE, France
[2]University of Melbourne, Faculty of veterinary and agricultural sciences, Victoria, Australia
[3]ANSES, UMR Mycoplasmose of ruminants, Lyon, France

Backgrounds

In recent years, antibiotic-resistance (AbR) has been increasingly observed in mycoplasmas, a large group of wall-less bacteria that comprises several important ruminant pathogens. In bacteria, two mechanisms are responsible for AbR emergence: acquisition of chromosomal mutations and/or horizontal gene transfer (HGT).

Objectives

The discovery of massive conjugative gene transfers in the ruminant pathogen *Mycoplasma agalactiae* radically changed how we viewed these minimal bacteria. This prompted us to address the impact of this phenomenon in mycoplasma AbR acquisition using *M. agalactiae* and enrofloxacin (Enro) as model.

Methods

We first isolated spontaneous *M. agalactiae* $Enro^R$ mutants by serial *in vitro* passages, in increasing Enro concentration. Mating experiments were then conducted using a mutant having the highest $Enro^R$ level as donor and an $Enro^S$ strain as a recipient.

Conclusions

A sequential accumulation of mutations was observed in 4 genes distributed in 3 genomic loci: *gyrA* and *gyrB*, encoding the DNA gyrase and *parC* and *parE*, encoding the topoisomerase IV for spontaneous mutation experiments. Mating experiments resulted in $Enro^R$ transconjugants and revealed the simultaneous transfer, in a single chromosome, of distant loci carrying the mutated target genes.
Since most mycoplasmas are deprived of plasmids, this study comforts the importance of chromosomally mediated AbR in mycoplasmas, with cumulative and sequential mutations correlating with the level of AbR. More importantly, our data point towards HGT as a major contributor and an accelerator of AbR acquisition in mycoplasmas by allowing the concomitant transfer of multiple dispersed mutations in one single event.

FEMS7-2002
Physiology / Biochemistry / Molecular Microbiology

PHENOTYPIC SWITCHING IN PSEUDOMONAS FLUORESCENS SBW25

G. Ferguson[1], P. Remigi[2], J. Gallie[3], P. Rainey[4]
[1]Massey University Auckland, Institute of Natural and Mathematical Sciences, Auckland, New Zealand
[2]Massey University Auckland, Institute for Advanced Study, Auckland, New Zealand
[3]Max Planck, Institute for Evolutionary Biology, 24306 Plön, Germany
[4]École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris ESPCI ParisTech, Laboratoire de Génétique de l'Evolution, Paris, France

Backgrounds

When challenged with repeated selective cycles through two contrasting environments, the bacterium *Pseudomonas fluorescens* SBW25 evolved, *de novo*, the ability to switch repeatedly between two bistable states – one where cells secrete an extracellular colanic acid-like capsule (Cap+), and an ancestral, uncapsulated state (Cap-). Subsequent characterisation showed the switch to be epigenetic, though underpinned by mutation at the start of the pyrimidine biosynthesis pathway.

Objectives

Taking a genetic approach, we sought to identify the ultimate cause of bistability.

Methods

Exploration of the genotype-phenotype map through a combination of transposon mutagenesis screens, reevolution and genome resequencing, transcriptome analysis, and gene deletion revealed a complex network of 'players' whose activities modulate Cap-/Cap+ switching.

Conclusions

Among these are the Gac/Rsm signalling pathway and the stringent response, specifically, RpoD and the nucleotide alarmone ppGpp, recently shown to underpin another bistable phenotype – that of antibiotic 'persisters'. Moreover, several lines of evidence indicate that expression of ribosome proteins is up-regulated in the switchers (Cap+) compared to wild-type cells. We present evidence in support of aberrant regulation of ribosome production as the underlying cause of Cap+/Cap- bistability in SBW25 'switcher' mutants.

FEMS7-1269
Physiology / Biochemistry / Molecular Microbiology

MAMMALIAN AKT PROTEIN KINASE OVERRUNS SLM1/2-YPK1/2-TORC2 SIGNALING IN SACCHAROMYCES CEREVISIAE

T. Fernandez-acero Bascones[1], *I. Rodriguez-escudero*[1], *V. Jimenez Cid*[1], *M. Molina Martin*[1]
[1]*Facultad De Farmacia- Universidad Complutense De Madrid, Microbiologia II, Madrid, Spain*

Backgrounds

Akt is a mammalian protein kinase involved in the regulation of key cellular processes and its up-regulation is commonly related to tumorigenesis. It is recruited to the plasma membrane (PM) by PIP_3, a lipid second messenger generated in response to extracellular stimuli by phosphoinositide 3-kinase (PI3K), and further activated through phosphorylation by the PDK1 and TORC2 kinases. An analogous mechanism has been reported for its essential *S. cerevisiae* counterparts Ypk1/2, which are recruited to the PM by the PIP_2-interacting proteins Slm1/2 and activated by yeast orthologues Pkh1/2 and Tor2. We have previously reported that *GAL1*-driven co-expression in yeast of PI3K and Akt leads to growth inhibition. Akt activation in yeast depends on both PM recruitment by ectopic PI3K-generated PIP_3 and phosphorylation by Pkh1/2, and that toxicity relies on severe PM overgrowth.

Objectives

We aimed to characterize the subcellular effects and signaling events occurring in yeast upon Akt stimulation.

Methods

We have used fluorescence and electronic microscopy to characterize membrane structures, transcriptomics to analyze the response to Akt activation and phosphorylation assays to identify Akt substrates in yeast.

Conclusions

We have found that Akt-derived membrane structures are enriched in actin patches, phosphatidylserine, PIP_2 and cell wall deposits in yeast cells. Overexpression of Akt leads to an oxidative stress transcriptional response and is able to complement the lack of Ypk1/2, causing the hyperphosphorylation of the PIP_2-effector, Slm1, thus indicating that activation of mammalian Akt in yeast is short-circuiting the normal function of the Slm1-Ypk1/2-TORC2 pathway in the maintenance of PM homeostasis.

FEMS7-3031
Physiology / Biochemistry / Molecular Microbiology

FATTY ACIDS AS FRIEND OR FOE: CONTRIBUTION OF VACJ AND FADL OUTER MEMBRANE PROTEINS TO NONTYPABLE HAEMOPHILUS INFLUENZAE INTERPLAY WITH THE HUMAN AIRWAYS

A. Fernández-Calvet[1], I. Rodríguez-Arce[1], G. Almagro[2], R. Conde-Alvarez[3], J. Moleres[1], S. Martí[4,5], L. Caballero[1], J.E. Yuste[5,6], J.A. Bengoechea[7], J. Garmendia[1,5]
[1]Instituto de Agrobiotecnología, Sanidad Animal, Mutilva, Spain
[2]Instituto de Agrobiotecnología, Metabolismo de Carbohidratos, Mutilva, Spain
[3]Universidad de Navarra, Microbiology and parasitology, Pamplona, Spain
[4]Hospital Universitari de Bellvitge IDIBELL, Microbiology, Barcelona, Spain
[5]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias, CIBERES, Madrid, Spain
[6]Centro Nacional de Microbiología ISCIII, Bacteriology- mycology and parasitology, Majadahonda, Spain
[7]Queen's University Belfast, Centre for Experimental Medicine, Belfast, United Kingdom

Backgrounds

Nontypable *Haemophilus influenzae* (NTHi) is an opportunistic pathogen associated to exacerbations of chronic obstructive pulmonary disease (COPD), a progressive irreversible airflow limitation accompanied by emphysema, fibrosis, inflammation and mucus hypersecretion. Fatty acid metabolites are key molecular mediators of COPD. Thus, NTHi may encounter free fatty acids in its colonizing niche. NTHi contains the *fadL* gene, encoding a fatty acid outer membrane (OM) transporter, but lacks a complete b-oxidation pathway, and a bactericidal effect of polyunsaturated fatty acids has been described for this pathogen. Conversely, NTHi contains all fatty acids and phospholipids (PL) biosynthetic genes, and *vacJ*/*mlaA*-*mlaBCDEF*, an intermembrane PL trafficking system serving to prevent PL accumulation in the OM, being VacJ/MlaA an OM lipoprotein. Mla mutant suppression by phospholipases (Pld) may result in production of free fatty acids that, in turn, could be taken by FadL, therefore functionally relating VacJ and FadL. In NTHi, Plds distribution is unknown, fatty acids uptake has not been shown, and VacJ contributes to virulence. We hypothesize that fatty acids uptake may not be fruitful to NTHi; also, VacJ and FadL may modulate bacterial fatty acid composition and/or interaction dynamics with exogenous hydrophobic molecules.

Objectives

Analysis of FadL and VacJ contribution to NTHi-host interplay.

Methods

NTHi *vacJ* and *fadL* mutant strains were generated and characterized in terms of growth, lipidic composition, lipid A structure, resistance to hydrophobic antimicrobials, airway infection *in vitro* and *in vivo*.

Conclusions

The results obtained highlight the importance of maintaining the bacterial surface integrity as a whole during NTHi airway infection.

FEMS7-1828
Physiology / Biochemistry / Molecular Microbiology

DNA-BINDING ACTIVITY OF CLOSTRIDIUM DIFFICILE FUR DEPENDS ON ITS CYSTEINES REDOX STATE

A. Fernández-Otal[1], M.L. Peleato[1], M.F. Fillat[1], Á. Lanas[2], M.T. Bes[1]
[1]Faculty of Sciences and Biocomputation and Complex Systems Physics Institute BIFI- University of Z aragoza,
Department of Biochemistry and Molecular and Cell Biology- Biochemical- Biophysical and Computati onal Biology Group - CSIC Joint Unit, Zaragoza, Spain
[2]Aragón Health Research Intitute- IIS, Department of Medicine- University of Zaragoza, Zaragoza, Spain

Backgrounds

Clostridium difficile, a gram-positive, anaerobic bacterium is regarded as the major cause of antibiotic associated colitis. In recent years, *C. difficile* antibiotic-resistance strains have appeared and different studies link such resistance with altering both the expression of redox proteins and iron metabolism. Iron homeostasis in bacteria is highly controlled by the metal-dependent transcriptional regulator Fur (Ferric uptake regulator). Apart from its well-known role in iron homeostasis, it has also been described as a redox sensitive protein in some microorganisms.

Objectives

The aim of this work was to study the redox characteristics of *C. difficile* Fur (*Cd*Fur) and their implication in the mechanism of action of this protein.

Methods

We overexpressed in *Escherichia coli* and purified by Immobilized Metal ion Affinity Chromatography the *Cd*Fur repressor and several cysteine mutants. We also analyzed the biochemical features of the native protein and its seven single cysteine mutants. Gel retardation assays with selected promoter genes (EMSA) were used to verify the biological activity of the recombinant proteins under different conditions *in vitro*. Furthermore, a modified FURTA (Fur titration assay) analysis was set up to determine Fur activity *in vivo.*

Conclusions

The *Cd*Fur protein showed two forms, a reduced one showing DNA-binding activity to its own promoter and an oxidized inactive form. Conversion between them was dependent on the formation of a disulphide bridge. Moreover, a single cysteine residue was essential for the binding of the protein to the corepressor metal.

FEMS7-1864
Physiology / Biochemistry / Molecular Microbiology

HIGH-THROUGHPUT SCREENING FOR MODULATORS OF CLOSTRIDIUM DIFFICILE FUR ACTIVITY

A. Fernández-Otal[1], M.L. Peleato[1], M.F. Fillat[1], J. Sancho[1], Á. Lanas[2], M.T. Bes[1]
[1]Faculty of Sciences and Biocomputation and Complex Systems Physics Institute BIFI- University of Z aragoza,
Department of Biochemistry and Molecular and Cell Biology- Biochemical- Biophysical and Computati onal Biology Group - CSIC Joint Unit, Zaragoza, Spain
[2]Aragón Health Research Institute IIS Aragón, Department of Medicine- University of Zaragoza, Zaragoza, Spain

Backgrounds

Clostridium difficile is a Gram-positive, spore-forming, anaerobic, intestinal bacterium and a common cause of antibiotic-associated colitis. The development of antimicrobials is critical in this time of increasing emergence of hypervirulent strains that display resistance to antibiotics. Several studies relate resistance to some antibiotics with altering iron metabolism in this pathogenic bacterium. The canonical bacterial repressor responsible for iron-dependent regulation is the ferric uptake regulator (Fur). *C. difficile* has a functional Fur (*Cd*Fur) implicated in the regulation of a subset of genes including virulence genes. Therefore *Cd*Fur constitutes an attractive target for the development of new antimicrobial compounds against this pathogen.

Objectives

Identification of bioactive compounds that may specifically modulate Fur activity in *Clostridium difficile*.

Methods

A high-throughput screening methodology based on thermal denaturation of the purified protein was used to identify small molecules of a chemical library that modulate *Cd*Fur activity. The effect of these compounds on the DNA-binding activity of *Cd*Fur *in vitro* was tested using gel retardation assays.

Conclusions

Several compounds from the chemical library were able to bind to *Cd*Fur homolog increasing protein stability. Some of them enhanced *Cd*Fur affinity for its own promoter and other gene promoters involved in iron acquisition by this pathogen.

FEMS7-1376
Physiology / Biochemistry / Molecular Microbiology

CHANGES IN LIPID A HYDROXYLATION AND LPXO EXPRESSION ARE REGULATED BY THE GLOBAL REGULATOR FNR IN SALMONELLA ENTERITIDIS

P. Fernández[1], F. Velásquez[1], J. Ortega[1], C.A. Silva[2], H. Garcias-Papayani[1], F. Amaya[1], C.A. Santiviago[1], S.A. Álvarez[1]
[1]Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Santiago, Chile
[2]Tufts University, Department of Molecular Biology and Microbiology, Boston- MA, USA

Backgrounds

Lipid A is the bioactive component of lipopolysaccharide and presents a dynamic structure that undergoes modifications in response to environmental changes. Lipid A hydroxylation, catalyzed by LpxO, is important for *Salmonella* virulence and is dependent on oxygen as substrate. Noteworthy, a regulatory role for oxygen availabity on *lpxO* expression has not been established. The adaptation of *Salmonella* to anaerobiosis involves changes in the expression of numerous genes, and the transcription factor FNR is a major regulator of this process.

Objectives

The aim of this work was to determine if *lpxO* expression and lipid A hydroxylation are regulated by the oxygen availability in *S.* Enteritidis, and the participation of FNR in this process.

Methods

We determined by mass spectrometry that lipid A hydroxylation is reduced when wild-type *S.* Enteridis is grown anaerobically, while this change is not observed in the case of a Δ*fnr* mutant, indicating that FNR regulates this process. Also, we demonstrated by qRT-PCR and the analysis of a *lacZ* transcriptional fusion that *lpxO* expression is higher when wild-type bacteria are grown aerobically than anaerobically. This differential expression pattern is not observed in the Δ*fnr* mutant strain. Finally, electrophoretic mobility shift assay revealed that FNR directly interacts with *lpxO* promoter region.

Conclusions

FNR binds to *lpxO* promoter and this would account for the oxygen-dependent changes observed in lipid A hydroxylation and in the expression of this gene in *S.* Enteritidis.

This work was supported by FONDECYT grants 1130225 and 1140754, and CONICYT fellowships 21140692 and 22151395

FEMS7-1180
Physiology / Biochemistry / Molecular Microbiology

THE TOPOLOGICAL ORGANIZATION OF THE CHROMOSOME OF STREPTOCOCCUS PNEUMONIAE REVEALED BY TRANSCRIPTOMIC ANALYSIS

M.J. Ferrandiz[1], *A.J. Martín-Galiano*[1], *A.G. de la Campa*[1]
[1]*National Centre for Microbiology- ISCIII, Unidad de Genética Bacteriana, Majadahonda- Madrid, Spain*

Backgrounds

DNA-supercoiling and the association of nucleoid-binding proteins compact bacterial chromosome in a way optimal for DNA transactions. The supercoiling level is maintained homeostatically by the opposing activities of relaxing DNA topoisomerases (topoisomerases I and IV) and by gyrase, which introduces negative supercoils. Changes in DNA-supercoiling orchestrated a transcriptomic response of the genome of *Streptococcus pneumonia, which* reveals topological reacting domains. Relaxation of DNA with the gyrase inhibitor novobiocin affects 36% of the genome, 68% of the differentially-expressed genes (DEGs) forming 15 domains (Ferrándiz, Nucleic Acids Res, 2010). Hyper-supercoiling achieved with seconeolitsine (a topoisomerase I inhibitor) affects 10% of the genome, 25% of the DEGs forming 12 domains (Ferrándiz, Nucleic Acids Res, 2016)**.** Domains detected under these opposing situations essentially overlapped

Objectives

To study the topological organization of the chromosome of *Streptococcus pneumoniae* under conditions of no global supercoiling alterations

Methods

RNA-seq, Chromatin Immunoprecipitation (ChIP), Bioinformatic analysis

Conclusions

The analysis of the transcriptomic response to subinhibitory concentrations of seconeolitsine plus novobiocin did reveal a response. DEGs accounted for 41% of the genome, 70% of them being located into 18 topological domains. These domains were larger, although overlapping those detected under supercoiling alterations caused by a single drug. In addition, a higher order organization, including the whole genome, with 9 domains containing an average of 227 genes was detected. These results will be correlated with those obtained by topoisomerase I and gyrase ChIP. These transcriptomic results support that the chromosome is organized into topological domains with fixed location

FEMS7-1287
Physiology / Biochemistry / Molecular Microbiology

ROLE OF THE CSRA POST-TRANSCRIPTIONAL REGULATORS IN PSEUDOMONAS SYRINGAE PV. TOMATO DC3000

M.D. Ferreiro García[1], J. Nogales[2], G.A. Farias[1], A. Olmedilla[3], M.T. Gallegos[1]
[1]Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas, Department of Soil Microbiology and Simbiotic Systems, Granada, Spain
[2]The Samuel Roberts Noble Foundation, Plant Biology Division, Ardmore- Oklahoma, USA
[3]Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas, Department of Biochemistry- Cell and Molecular Biology of Plants, Granada, Spain

Backgrounds

RsmA/CsrA proteins are post-transcriptional regulators widely distributed among gamma-proteobacteria. They alter translation, mRNA turnover, and/or transcript elongation by binding to the 5′ untranslated region (5'-UTR) and/or early coding regions of mRNAs. CsrA proteins are released from their target mRNAs upon being sequestered by the small regulatory RNAs rsmX, rsmY or rsmZ, whose expressions depend on the GacS/GacA two-component system. *Pseudomonas syringae* pv. tomato DC3000 (Pto DC3000) is a model pathogenic bacteria used to study plant-phytopathogen interactions. So far, the Gac-rsm regulatory pathway has been shown to control virulence, motility, production of secondary metabolites, carbon metabolism and quorum sensing in Pto DC3000. This strain encodes in its genome seven rsm regulatory RNAs: rsmX1-5, rsmY and rsmZ and five CsrA paralogs, three of which are well conserved among *Pseudomonas* species, *csrA1*, *csrA2* and *csrA3*.

Objectives

Although the possible components of the Gac-rsm pathway in Pto DC3000 are known, their physiological roles have not yet been established. Therefore the aim of this work is to elucidate the role of CsrA1, CsrA2 and CsrA3 in Pto DC3000 biology, paying particular attention to virulence-related phenotypes.

Methods

We have constructed deletion mutants and carried out their phenotypic characterization in different laboratory conditions (swimming, swarming, flagella production, syringafactin and alginate synthesis) and *in planta.*

Conclusions

CsrA1, CsrA2 and CsrA3 have non-redundant functions repressing alginate production, swimming and swarming motility, flagella and syringafactin synthesis and virulence in tomato plants. Gac-rsm regulation of those processes is exerted mainly through CsrA3, CsrA2 has a minor role, and CsrA1 does not intervene at all.

FEMS7-2439
Physiology / Biochemistry / Molecular Microbiology

COMPLEX REGULATORY NETWORK CONTROLS CHROMOSOME SEGREGATION IN STREPTOMYCETES

Ž. Filić[1], T. Paradžik[1], D. Jakimowicz[2], D. Vujaklija[1]
[1]Institut Ruđer Bošković, Division of Molecular Biology, Zagreb, Croatia
[2]Faculty of Biotechnology- University of Wrocław, Department of Molecular Microbiology, Wrocław, Poland

Backgrounds

Streptomyces species are multicellular filamentous Actinobacteria, producers of numerous secondary metabolites used in biotechnology and medicine. Streptomycetes undergo complex cellular differentiation including formation of vegetative mycelium followed by aerial mycelium and then spores. The spores are produced at the tip of aerial filaments and help these multicellular bacteria to disperse in the environment. These processes require a complex gene regulatory network of proteins ensuring the proper chromosome organization and partitioning of DNA into pre-spore compartments before septa closure. Various proteins are known to be involved in chromosomal segregation during sporulation and some of them were used in this study.

Objectives

Our goal was (I) to construct double and triple mutants of ssbB, parB and smc, (II) to examine how these mutations affect chromosome segregation during sporulation in order to elucidate whether these genes participate in the same or various regulatory cascades.

Methods

PCR targeting system and conjugation were used to construct various strains mutated in selected genes. These strains were examined for growth and sporulation defects. Fluorescence microscopy was used to determine the efficiency of DNA distribution in spores compartments, spore size as well as the number of spores in areal hyphae.

Conclusions

The results revealed a severe chromosome segregation defect in SsbB depleted *S. coelicolor* mutant, indicating the importance of SsbB protein for the reproductive stage of growth. In double and triple mutants more pronounced defects in chromosome segregation during sporulation occured than previously reported for any of the parental strains indicating that analysed genes participate in different regulatory network.

FEMS7-1658
Physiology / Biochemistry / Molecular Microbiology

REDOX STATUS OF C93 IN FURB FROM ANABAENA SP. PCC 7120 MODULATES BINDING OF REGULATORY ZINC AND ITS INTERACTION WITH TARGET DNA

V. Calvo Sein-Echaluce[1], I. Yruela[2], A. Velázquez-Campoy[3], M.T. Bes[1], M.L. Peleato[1], M.F. Fillat[1]
[1]Faculty of Sciences and Biocomputation and Complex Systems Physics Institute BIFI- University of Z aragoza,
Department of Biochemistry and Molecular and Cell Biology- Biochemical- Biophysical and Computati onal Biology Group - CSIC Joint Unit, Zaragoza, Spain
[2]Aula Dei Experimental Station EEAD-CSIC and Complex Systems Physics Institute BIFI,
Biochemical- Biophysical and Computational Biology Group - CSIC Joint Unit, Zaragoza, Spain
[3]Complex Systems Physics Institute BIFI and Fundación ARAID- University of Zaragoza,
Biochemical- Biophysical and Computational Biology Group - CSIC Joint Unit, Zaragoza, Spain

Backgrounds

FurB (Zur) is the FUR (ferric uptake regulator) paralog present in *Anabaena* sp. PCC 7120 that controls zinc homeostasis by binding to DNA in a zinc-dependent manner. Most Zur proteins contain a structural zinc atom that is tretracoordinated by four highly-conserved cysteine residues also present in FurB from *Anabaena* sp. PCC 7120. Apart from the structural atom, most Zur proteins are able to bind one to two additional zinc atoms, involved in DNA binding and protein dimerization.

Objectives

To elucidate the relationship among the zinc-binding ability of *Anabaena* sp. PCC 7120 FurB, the redox environment and the activity of this protein.

Methods

Zinc content in recombinant FurB was determined by Inductively Coupled Plasma spectrometry (ICP). Homology modelling was driven with Modeller using the structures of *H. pylori* Fur, *M. tuberculosis* FurB/Zur and *S. coelicolor* Zur as templates and analysed with PyMol 1.4.1. Interaction of FurB with zinc was studied by isothermal titration calorimetry (ITC). Contribution of cysteine residues to FurB activity was determined by site-directed mutagenesis and FurB activity was assessed by electrophoretic mobility shift assays (EMSA).

Conclusions

Recombinant FurB was able to bind three zinc atoms under reducing conditions. The redox state of cysteine C93 influences the binding of the second regulatory zinc atom and in turn modulates the affinity for a specific DNA target.

FEMS7-1174
Physiology / Biochemistry / Molecular Microbiology

IDENTIFICATION OF STRESS-RESPONSIVE MICRORNAS IN PLANT-MICROBE INTERACTIONS

M.M. Finetti Sialer[1], D. De Paola[1], K. Ogata Gutierrez[2], D. Zúñiga-Dávila[2], C. Chumpitaz-Segovia[2]
[1]Institute of Biosciences and Bioresources IBBR-CNR, DISBA, Bari, Italy
[2]Laboratorio de Ecología Microbiana y Biotecnología- Universidad Nacional Agraria La Molina, Dpto. de Biología, Lima, Peru

Backgrounds

Non-coding small RNAs (sRNAs) regulate gene expression in plant development and stress response. Different sRNA, in particular microRNAs (miRNAs) are principal mediators of the plant immune system. Roots endophytic bacteria and fungi affect crops performance acting as key soil factors underpinning ecosystem functioning and determining the properties of associated biota.

Objectives

We aimed at identifying plant miRNAs involved in the tri-trophic interaction of a beneficial bacterium (*Pseudomonas* sp.) and *Solanum lycopersicum* challenged with the pathogenic fungus *Alternaria alternata*, through comparative analyses carried out in different plant-microorganism associations.

Methods

sRNA profiling was performed on 3-week-old plants of *S. lycopersicum* plants treated with *Pseudomonas* sp. (Ps155) or challenged with the pathogen, alone or in combination, or non-treated as control. RNA extraction and library construction was carried out in two biological replicates per treatment and sequenced by Illumina technology. The sRNA libraries were aligned to the tomato genome (release 2.40). Reads (100% matching) were mapped onto the miRBase release 21.0 to identify known miRNAs. Differential miRNA expression (DE) analysis was based on DESeq2.

Conclusions

334 known tomato miRNAs were identified in the sRNA libraries, of which near 50% were shared across libraries. The four treatments were evaluated in pairwise comparisons to gain evidence for their DE among treatments. Statistical analyses highlighted 27 differential regulated miRNAs. These DE targeted different tomato genes including transcription and growth-regulating factors based on computational target prediction. Data provide a comparative analysis of changes in the small RNA profiles and the putative gene targets they control.

FEMS7-1056
Physiology / Biochemistry / Molecular Microbiology

STRIPPING BACILLUS ANTHRACIS' PROTECTIVE COAT

A. Fioravanti[1], W. Jonckheere[1], E. Pardon[1], J. Steyaert[1], H. De Greve[1], H. Remaut[1]
[1]VIB/Vrije Universiteit Brussel, Structural Biology Research Center, Brussels, Belgium

Backgrounds

The Gram-positive spore forming bacterium *Bacillus anthracis* is the aetiological agent of anthrax. *B. anthracis* spores' enter the body via skin lesions, lungs or a gastrointestinal route and germinate within the host, giving rise to the vegetative form of the bacillus. The surface of the vegetative bacterium is covered by a two-dimensional protein array known as "S-layer". Two mutually exclusive S-layers sequentially appear at the cell surface in a growth phase-dependent manner (Sap-layer: exponential growth; EA1-layer: stationary phase).

Objectives

Previous studies showed that in laboratory condition, deletion of Sap led to cell division defects causing a chain length phenotype, suggesting that Sap is a promising candidate for the development of new strategies to fight anthrax disease.

Methods

The self-assembling characteristic of S-layer proteins hampers their ease of handling under non-denaturing conditions and has hitherto proven prohibitive for structural and biophysical characterisation.In order to address the self-polymerisation issue, we optimised the expression and purification of native monomeric Sap to allow immunisation of llamas with the aim of obtaining Nanobodies (Nbs) specific for the monomeric form of the protein.

Conclusions

A systematic search identified a set of Nbs that stabilise Sap and allowed its crystallisation and structure determination, showing an extended structure composed of 6 immunoglobulin-like domains. In addition, we identify Nbs able to inhibit Sap *in vitro* polymerisation. These inhibitory Nbs will be candidates for *in vivo* inhibition assays and evaluation for their infection attenuating ability.

FEMS7-0492
Physiology / Biochemistry / Molecular Microbiology

COMPLETE GENOME SEQUENCE AND METHYLOME ANALYSIS OF BEGGIATOA LEPTOMITOFORMIS STRAINS D401 AND D402

A. Fomenkov[1], Z. Sun[2], T. Vincze[3], M. Orlova[4], B. Anton[1], M. Grabovich[4], R. Roberts[1]
[1]New England Biolabs, Genome biology, Ipswich, USA
[2]New England Biolabs, Bioinformatic, Ipswich, USA
[3]New England Biolabs, IT, Ipswich, USA
[4]Voronezh State University, Biology, Voronezh, Russia

Backgrounds

The taxonomy of *Beggiatoa* genus is still a work in progress. Despite many morphotypes of the *Beggiatoa* genus having been described in the literature, only one species, *B. alba* has been validated until now. We described a second species, *B. leptomitoformis.* Two strains of *B. leptomitoformis* D401 and D402 have been isolated from different regions of Russia. They differ in their morphology and physiology especially their ability to growth lithotrophically in the presence of thiosulfate. While *B. leptomitoformis* D402 is able to accumulate elemental sulfur, strain D401 cannot.

Objectives

We performed genomic sequencing of these two strains using the PacBio SMRT platform and assembled the reads into two complete circular genomes.

Methods

The advantage of the PacBio sequencing platform is its ability not only assemble complete closed genome, but to detect the epigenetic state of the sequenced DNA, which allows for the identification of modified nucleotides and the corresponding motifs in which they occur.

Conclusions

Both genome sequences have been deposited in GenBank with accession numbers CP018889 and CP012373 respectively. Surprisingly these two genomes showed almost 90% homology. The preliminary analysis of several thiosulfate oxidation and autotrophic assimilation of CO_2 metabolic operones in both stains did not revel any noticeable differences.
Thirteen DNA methyltransferase recognition motifs were found. They include one m4C and nine m6A modifications that were detected by direct SMRT sequencing and an additional three m5C motifs were detected in Tet2 treated DNA. The motifs were then matched with methyltransferase genes in the genome, and the results have been deposited in REBASE .

FEMS7-2279
Physiology / Biochemistry / Molecular Microbiology

CSRA, UCPA AND PFKA : MORE ALLOSTERIC REGULATION ?

N. Fraikin[1]
[1]*Free University of Brussels ULB, Génétique et Physiologie Bacterienne, Gosselies, Belgium*

Backgrounds

CsrA is a global regulator that modulates carbon distribution between different genetic programs in bacteria. It primarily regulates social behaviors, motility and metabolism. CsrA regulates the expression of several genes at the post-transcriptional level, by binding their transcripts and modulating their stability. It has been known for more than 20 years that glycolysis is impaired in a csrA mutant. However, subsequent works failed to demonstrate direct regulation of glycolytic enzymes by CsrA, suggesting that CsrA regulates glycolysis indirectly. Our lab has been lately interested in UcpA, a protein of unknown function showed to be an excellent candidate gene to be negatively regulated by CsrA. Subsequent data suggest that UcpA could be a regulator of glycolytic activity.

Objectives

We are studying the role of UcpA in the CsrA-mediated regulation of glycolysis, to show whether UcpA regulates glycolysis and how this regulation happens.

Methods

We showed that UcpA and PfkA (Phosphofructiokinase A, a glycolytic enzyme regulated by many allosteric effectors) form a complex in vivo and in vitro. Deleting ucpA in a csrA mutant partially suppresses some phenotypes caused by the deletion of csrA on glycolytic carbon sources (growth defect, hexose phosphate accumulation).

Conclusions

Our results suggest a inhibitory function of UcpA over PfkA. Since UcpA might be negatively regulated by CsrA, this might explain how a csrA mutant is defective for glycolytic activity.

FEMS7-1774
Physiology / Biochemistry / Molecular Microbiology

STRUCTURE AND ANTIMICROBIAL RESISTANCE OF NEISSERIA GONORRHOEAE IN COMUNIDAD VALENCIANA AND CATALONIA (SPAIN) BASED ON WHOLE-GENOME DATA

C. Francés-Cuesta[1], A. Fabregat-Bolufer[2], J. Serra-Pladevall[3], J. Colomina[2], A. Andreu[3], T. Pumarola[3], F. González-Candelas[1,4]
[1]Unidad Mixta Infección y Salud Pública FISABIO-CSISP/Univ. Valencia-I2SysBio, Genomic and Health, Valencia, Spain
[2]Hospital Universitario de La Ribera, Servicio de Microbiología, Alzira- Valencia, Spain
[3]Hospital Vall d'Hebron, Servicio de Microbiología, Barcelona, Spain
[4]CIBER en Epidemiología y Salud Pública, CIBERESP, Valencia, Spain

Backgrounds

Gonorrhea is the second most common bacterial sexually transmitted disease and its incidence increases every year worldwide. This, together with the growing number of antimicrobial resistance (AMR) isolates, have made *Neisseria gonorrhoeae* (NG) a threat to public health.

Objectives

The aim of the study was to perform an analysis of genomic variation in two sets of clinical samples of NG from two Spanish regions, Comunidad Valenciana (CV) and Catalonia, to compare their genetic structure and pattern of antibiotic resistance.

Methods

We analyzed 220 strains of NG, 127 from CV and 93 from Catalonia. These samples were sequenced using Illumina NextSeq technology. Sequences types (STs) were obtained using SRST2. Complete genome sequences were assembled *de novo* and annotated in order to compare the genetic features of each strain. These data were used for phylogenetic inference by maximum likelihood methods and to compare the diversity of NG isolates of these two regions.

Conclusions

We found 39 different STs, of which 13 were present in both regions and 13 were exclusive to each. 2 unidentified STs were also found in each of the study regions. The most prevalent types were ST1901, ST7363, and ST9363 (>57% in CV, >46% in Catalonia). Most strains were resistant to quinolones and penicillin in both regions. However, sensitivity to cephalosporins was high and no resistance was found to spectinomycin. Some resistance determinants, such *penA* (n=54), TEM-1D (n=30), and *tetM* (n=27), were detected. Further studies are necessary to determine all the possible causes of AMR acquisition in NG.

FEMS7-2294
Physiology / Biochemistry / Molecular Microbiology

ROLE OF THE GLUTAMINE-DEPENDENT ACID RESISTANCE SYSTEM OF BRUCELLA SPP. IN RESPONSE TO EXTREME ACID STRESS

L. Freddi[1], M.A. Damiano[1], E. Pennachietti[2], S. Köhler[1], D. De Biase[2], A. Occhialini[1]
[1]IRIM, CNRS, Montpellier, France
[2]Dipartimento di Scienze e Biotecnologie Medico-Chirurgiche, Sapienza Università di Roma, Latina, Italy

Backgrounds

The glutamate-dependent decarboxylase (GAD) system is the most potent acid resistance system (AR2) described in *Escherichia coli.* It consists of a glutamate decarboxylase enzyme and a glutamate/GABA antiporter. Our group showed that the GAD system is also operative in newly described and atypical *Brucella* species, among which *Brucella microti*. Recently, an AR2-Q system, based on deamination of glutamine to glutamate by the glutaminase YbaS, was also described in *E. coli*. In the genome of *Brucella*, a *glsA* gene homologous to *ybaS* is located just upstream and in same orientation as the *gadB* and *gadC* genes encoding the GAD system.

Objectives

Using a panel of genus-representative strains, our aim was to demonstrate the existence of a functional AR2-Q system in *Brucella* spp and its contribution to survival at an extreme acid stress.

Methods

In contrast to the classical *Brucella* species studied, only the new and atypical strains were found AR in the presence of glutamine and glutamate. By RT-PCR experiments, the genes of the *gadBC-glsA* locus were found expressed as an operon. Functional complementation of a *glsA* mutant of *B. microti* with the *ybaS* gene of *E. coli* demonstrated strong homologies between the 2 enzymes. Acid resistance phenotypes of mutant strains of *B. microti* confirmed the role of the AR2-Q system in extreme glutamine-dependent AR at pH 2.5.

Conclusions

The GAD and AR2-Q systems are functional in new and atypical species of *Brucella*. This study provides insight into the adaptation of these strains to extremely acidic environments.

FEMS7-3244
Physiology / Biochemistry / Molecular Microbiology

DETECTION AND IDENTIFICATION OF MYCOBACTERIUM SPECIES BY TWO DIFFERENT MOLECULAR METHODS COMPARING WITH CULTURE

A. gaber[1], E. elsawy[1], H. hamed[1]
[1]urology & nephrology center, microbiology, mansoura, Egypt

Backgrounds

Tuberculosis still remains a worldwide public health problem. Its causative agent is *Mycobacterium tuberculosis* which causes death in developing countries. Nontuberculous mycobacteria (NTM) are responsible for the majority of mycobacterial infections in resource-rich countries, where tuberculosis is not endemic.

Objectives

With the recent global issue of mycobacterial infections, it is necessarily to find rapid, sensitive, and specific diagnostic methods for the detection and identification of *M. tuberculosis* and NTM in clinics The traditional diagnosis of mycobacterial infection are culture-based identification method. Direct examination with microscope.

Methods

140 urine samples, Ziehl-Neelsen Acid Fast staining,culture by VersaTREK were performed. DNA was extracted using High Pure Template Purification Kit .The purified chromosomal DNA was used for PCR and microarray analysis. For PCR, the primers were designed depending on the DNA sequence alignments of the mycobacterial 16S rRNA conservative regions. The amplified DNA is 1350 bp. For microarray analysis, The LCD array kit (Myco ^{Direct} 1.7) was manufactured by Chipron GmbH, (Germany) and designed for identification of *Mycobacterium tuberculosis* complex and other Nontuberculous mycobacteria (NTM). It is based on a PCR amplification of rRNA gene region with 225-265 bp , and a 126 bp fragment from the IS*6110* element.
80 individuals gave positive mycobacterial culture test. 54 individuals were positive results by Ziehl-Neelsen staining. Out of the 80 culture positive samples, only 77 exhibited PCR true positive results. The LCD array analysis showed that the 80 positive cultures from urine specimens contains 10 *Mycobacterium tuberculosis,*30 *Mycobacterium kansasii* , 17 *Mycobacterium celatum*, 9 *Mycobacterium gordonae*,8 *Mycobacterium chelonae*, and 6 *Mycobacterium phlei*.

Conclusions

The simple one step exhibited high sensitivity and specificity would give an encouragement for MTB and NTM rapid diagnosis. Also, it is recommended to use the LCD-microarray for the mycobacterial species identification as a rapid and highly sensitive diagnosis rather than classical methods.

FEMS7-1404
Physiology / Biochemistry / Molecular Microbiology

EMM TYPE DISTRIBUTION OF MACROLIDE RESISTANT GROUP A STREPTOCOCCI ISOLATED FROM PATIENTS WITH ACUTE PHARYNGITIS IN SERBIA (2008-2014)

I. Gajic[1], V. Mijac[1], L. Ranin[1], D. Kekic[1], S. Popovic[1], A. Smitran[2], N. Opavski[1]
[1]Medical Faculty, Institute of microbiology and immunology, Belgrade, Serbia
[2]Medical Faculty, Microbiology, Banja Luka, Bosnia - Herzegovina

Backgrounds

The prevalence of erythromycin resistance of pharyngeal group A streptococci (GAS) in Serbia increased significantly from 2.41% in 1990s to 12.5% in 2008.

Objectives

The aims of this study were to determine the prevalence of macrolide-resistant GAS (MRGAS) during 2011-2014 and to describe changes in distribution and dynamics of resistant *emm* types over time.

Methods

A total of 2180 GAS isolated from patients with acute pharyngitis from 2011 to 2014 were included in this study. Susceptibility to erythromycin was determined by disk diffusion test. The sequence-typing was used for MRGAS *emm* assignment. Obtained data were compared with 3891 previously characterized GAS recovered in 2008/2009.

Conclusions

A steady decline in macrolide resistance was recognized from 2008 to 2014 in Serbia (2008/2009-12.5%; 2011/2012-9.6%; 2013/2014-9.4%). A total of 13 *emm* types of MRGAS were detected in the study period. The most frequent *emm* types in 2008/2009 (*emm*75, *emm*77, *emm*12) and 2011/2012 (*emm*75, *emm*12, *emm*1) accounted for 94.7% and 83.7%, respectively. In 2013/2014 *emm*12, *emm*89 and *emm*75 comprised 70.4% of all MRGAS. The genotype *emm*77, the third common in 2008/2009 (14.6%), was not detected in 2011/2012 while in 2013/2014 accounted for just 3.7% of all MRGAS. Genotype *emm*89 was identified for the first time in Serbia in 2011/2012, and its prevalence increased significantly more than 3-fold in 2013/2014, being the second most common type.
The decrease of macrolide resistance during 7-year period might be related to a slight change in the distribution of *emm* types in the community.

FEMS7-1771
Physiology / Biochemistry / Molecular Microbiology

IN VIVO INTERACTIONS OF THE TWO COMPONENT SYSTEM EUPK/EUPR IN THE HALOPHILIC BACTERIA CHROMOHALOBACTER SALEXIGENS: A CROSS-REGULATED SYSTEM

R.M. García-Valero[1], M. Argandoña[1], D. Wolf[2], J.J. Nieto[1], T. Masher[2], C. Vargas[1]
[1]Universidad de Sevilla, Microbiología y Parasitología, Sevilla, Spain
[2]Technische Universität TU Dresden, Institute of Microbiology, Dresden, Germany

Backgrounds

Chromohalobacter salexigens is a halophilic bacterium able to grow in a wide range of salinities (0.5 to 3 M NaCl in minimal medium). Its main osmoadaptation strategy is the cytoplasmic accumulation of the biostabilizers ectoines, two compounds with great industrial interest. To coordinate these processes, the cells are equipped with different systems and mechanisms for osmosensing and signal transduction, that induce the cellular response necessary to adapt them to these conditions. These signal transduction pathways have not yet been described in depth in halophilic bacteria. Previously, we phenotypically characterized a two-component system, EupK/EupR, involved in the osmoadaptation and metabolism of this halophile, through the control of the synthesis, degradation and uptake of ectoines. This was also cross-linked with the regulation of its glucose catabolism.

Objectives

Analysis of *in vivo* protein-protein interaction between the histidine kinase EupK and its response regulator EupR in different conditions of salinity and carbon sources.

Methods

Construction of a tagged-protein expression vector based on the pMP92 plasmid. Expression under different salinity and carbon sources conditions of the tagged proteins. Purification and analysis of putative protein-protein interactions performed by a membrane-SPINE assay and co-inmuno precipitation in *C.salexigens*.

Conclusions

Preliminary results demonstrate the interaction between the response regulator and the histidine kinase and reveal the presence of other proteins that interact with this regulation system. From these data it can be suggested that EupK/EupR probably forms part of a more complex transcriptional regulatory network via a cross-regulated system.

Funding:BIO2015-63949-R(MINECO/FEDER, UE), Junta de Andalucía(P11-CV1-7293 MO), ERASMUS+, University of Sevilla PIF grant.

FEMS7-1229
Physiology / Biochemistry / Molecular Microbiology

FLUOROQUINOLONE RESISTANCE, DNA-CLEAVAGE, REACTIVE OXYGEN SPECIES PRODUCTION, AND PHAGE INDUCTION AFFECTS THE POST-ANTIBIOTIC EFFECT OF LEVOFLOXACIN IN STREPTOCOCCUS PNEUMONIAE

M.V. Valenzuela[1], M.J. Ferrándiz[2], A.G. de la Campa[2], M.T. García-Esteban[1]
[1]Universidad Complutense de Madrid, Microbiología III, Madrid, Spain
[2]Centro Nacional de Microbiología. Instituto de Salud Carlos III, Unidad de Genética Bacteriana, Majadahonda Madrid, Spain

Backgrounds

The fluoroquinolone levofloxacin (LVX) is currently used for treatment of pneumococcal infections. The post-antibiotic effect (PAE) has a clinical impact on antibacterial dosing regimens. LVX acts via the formation of DNA-LVX-topoisomerase complexes, with the subsequent generation of detrimental double-stranded DNA breaks. Its lethality is enhanced by the production of reactive oxygen species (ROS), via the up-regulation of genes that stimulate the Fenton reaction. In addition, LVX causes bacterial lysis by phage induction

Objectives

To study the factors get involved in the PAE of LVX

Methods

Susceptible and resistant pneumococcal strains were analyzed during the postantibiotic phase. Growth curves, level of chromosome fragmentation, and ROS production were determined. In addition, isogenic isolates with prophages inducible or non inducible by LVX were studied

Conclusions

Treatment of 1 h with LVX at 2.5× MIC induced EPAs between 0.22±0.05 and 1.41±0.21 h. Treatment with 10× MIC induced longer EPAs (between 0.56±0.11 and 2.06±0.35 h). EPA values were lower in LVX-resistant strains. In pneumococcal cultures treated with LVX (2.5× MIC, 1 h), after 4 hours of removing the antibiotic, bacteria viability increased by 10-fold, while ROS level decreased by 10-fold, and DNA breakage decreased by 7-fold. EPA induced in an isolate carrying an inducible prophage was 3-fold longer than in a non-inducible isolate. LVX induces significant EPAs in *S. pneumoniae*, which were related with the resistance of the strain, ROS production, DNA cleavage and presence of LVX-inducible prophages

FEMS7-1693
Physiology / Biochemistry / Molecular Microbiology

MEMBRANE MICRODOMAINS DISASSEMBLY AFFECTS STAPHYLOCOCCUS AUREUS VIRULENCE

E. García-Fernández[1], *D. López*[1]
[1]*Centro Nacional de Biotecnología, Biotecnología Microbiana, Madrid, Spain*

Backgrounds

The pioneering *fluid mosaic model* of the cell membrane has been reviewed in the past two decades due to the evidence that biological membranes contain diverse lipid species, which tend to segregate laterally into membrane microdomains. Eukaryotic cells organize proteins important for the correct functionality of numerous cellular functions into membrane microdomains or *lipid raft*. The integrity of *lipid rafts* is partly mediated by the activity of raft-associated scaffold proteins termed flotillins. Recently, it has been described that bacterial cells also compartmentalize cellular processes in functional membrane microdomains (FMM) that structurally resemble that of eukaryotic lipid rafts.

Objectives

Here we use the human pathogen *Staphylococcus aureus* to determine the composition, assembly and biological function of bacterial FMM.

Methods

Super resolution microscopy approaches have been used to identify structural determinants of FMM and examine the subcellular localization of flotillin. The constituent lipids and proteins associated to FMM were analyzed using UPLC-ESI-qTOF-MS and MS-LFQ respectively. The influence of perturbation of FMM was analyzed by *in vitro* and *in vivo* experiments.

Conclusions

A mutually guided interaction between flotillin and membrane saccharolipids organizes saccharolipid-rich membrane platforms that promote efficient oligomerization of protein interaction partners. We show that alterations in FMM organization lead to a severe and simultaneous perturbation of proteins complexes that are harbored in the FMM. We show how this can be used to inhibit the virulence potential of this pathogen and how it can be developed as a new antimicrobial therapy.

FEMS7-0942
Physiology / Biochemistry / Molecular Microbiology

CONFORMATIONAL LOCKING BY PHOSPHORYLATION AS A REGULATORY SWITCH FOR BACTERIAL TRANSLATION-ELONGATION FACTORS

A. Garcia-Pino[1]
[1]*Free University of Brussels ULB, Department of Molecular Biology, Gosselies, Belgium*

Backgrounds

Bacterial protein synthesis is intricately connected to metabolic pace. It responds to environmental stress through post-translational modifications of transcription and translation factors. Translation elongation factor Tu (EF-Tu) is among the most heavily modified. EF-Tu is methylated and phosphorylated in response to nutrient starvation upon entering stationary phase and its phosphorylation is a crucial step in the pathway towards sporulation.

Objectives

We have discovered that reversible phosphorylation of EF-Tu strongly affects its function as a translation elongation factor and is potentially a very important regulator of translation in general. Our data indicates that phosphorylation decouples the EF-Tu conformational cycle (open/closed states) from the control of nucleotides and P-EF-Tu remains trapped in an open conformation that precludes aa-tRNA binding. Our X-ray structures show that the bases of the inactivation are intricately related with the function of the switch I and II regions. In addition we use phosphomimetic mutations on other conserved phosphorylation sites to show that the mechanism of inactivation is likely general.

Methods

We performed in-depth studies on the behavior of pEF-Tu in relation to its multiple partners with a variety of structural and biophysical techniques (including thermodynamics, stopped-flow kinetics, SAXS and X-ray crystallography and smFRET). Only from the integration of all these data that combine structure and dynamics, it becomes apparent that the real effects of phosphorylation lay on the EF-Tu dynamics.

Conclusions

Overall these results could have potential implications to the way EF-Tu is further modified *in vivo* for instance during stationary phase to reduce the pace of translation

FEMS7-1977
Physiology / Biochemistry / Molecular Microbiology

REGULATION ON NITRATE RESPIRATION IN THERMUS THERMOPHILUS

L. Álvarez[1], C. Bricio[2], N. García-Quintáns[2], A. Blesa[2], I. Baquedano[2], M. Mecía[2], J. Berenguer[2]
[1]Umeå Centre for Microbial Research,
Laboratory for Molecular Infection Medicine Sweden- Department of Molecular Biology, Umeå, Sweden
[2]Centro de Biología Molecular "Severo Ochoa" CSIC-UAM, Molecular Biology, Madrid, Spain

Backgrounds

Denitrification capability is an unevenly distributed property in the extreme thermophile *Thermus thermophilus*, where strictly aerobic, nitrate respiring, and denitrificant strains exist. The corresponding genes are distributed in two gene clusters, one encoding nitrate respiration (NCE) and the other nitrite respiration (*nic*), located in a poorly conserved region of a megaplasmid and easy transferable both by natural competence and by transjugation, a new class of cell-to-cell DNA transfer mechanism. The denitrification genes are expressed in response to oxygen depletion and presence of the corresponding nitrogen oxide. However, no homologues of common regulators described for denitrification in other bacteria such as FNR, DNR or NarX/NarL two component systems are found in *T. thermophilus.*

Objectives

To identify and analyse the regulators that control the expression of the nitrite and nitric oxide reductases encoded by the *nic* cluster of *T. thermophilus*.

Methods

Mutants defective in putative transcription factors were subjected to expression assays using promoter vectors expressing thermostable beta-galactosidase. Pure native and mutant proteins were also subjected to *in vitro* EMSA assays.

Conclusions

Master regulators encoded by the NCE cluster are required for the expression of the promoters in the *nic* cluster. In addition, a regulator related to the NsrR NO-sensing protein of Gram positive bacteria was also required for nitrite respiration. In contrast to its homologues the *T. thermophilus* protein binds the target promoter in its oxidized form, allowing us to propose a global regulation mode for nitrite respiration in *T. thermophilus.*

This work has been funded by Spanish national project BIO2016-77031-R

FEMS7-1263
Physiology / Biochemistry / Molecular Microbiology

INVOLVEMENT OF THE SMALL RNA SUHB ON CARBON CATABOLITE REPRESSION OF TETRALIN DEGRADATION GENES IN SPHINGOPYXIS GRANULI STRAIN TFA

I. García-Romero[1], *E. Santero*[1], *B. Floriano*[1]
[1]*Andalusian Center for Developmental Biology,*
Pablo de Olavide University and Department of Molecular Biology and Biochemistry Engineering, Sevilla, Spain

Backgrounds

Sphingopyxis granuli strain TFA is able to use the organic solvent tetralin as the sole carbon and energy source. Structural *thn* genes are induced in the presence of tetralin by ThnR, a LysR-like transcriptional regulator, and ThnY, a ThnR co-activator. Besides, the expression of *thn* genes is under carbon catabolite repression (CCR) by a preferential carbon source, such as β-hydroxybutyrate (β-HB).

Objectives

The main objective is to stablish the role of a small RNA (sRNA) in *thn* genes expression control under CCR conditions.

Methods

Comparison of the global gene expression in tetralin- and β-HB-grown cells revealed the presence of a sRNA expressed preferentially in β-HB, which was annotated by Infernal software 1.1. Northern Blot and β-galactosidase assays confirmed the differential expression of this sRNA. The effect on CCR over *thn* genes of mutant lacking the sRNA was evaluated. Furthermore, its putative targets were detected by IntaRNA software and the interaction validated by RNA-RNA EMSA.

Conclusions

The sRNA is predicted as belonging to the family RF00519 (SuhB), which is highly conserved in α-proteobacteria. The *thn* genes are partially de-repressed in a mutant lacking SuhB under CCR conditions. Furthermore, the 5' UTR of *thnR* mRNA is identified as a target of SuhB in which would block the ribosomal binding site. Accordingly, ThnR levels are higher in the mutant strain. The available data so far indicate that SuhB is involved in CCR of *thn* genes.

FEMS7-1164
Physiology / Biochemistry / Molecular Microbiology

OPTIMIZATION OF A REPORTER ASSAY FOR SMALL-RNA TARGET VALIDATION IN A-PROTEOBACTERIA

N.I. García-Tomsig[1], J.I. Jiménez-Zurdo[1], M. Robledo[1]
[1]Estación Experimental del Zaidín- Consejo Superior de Investigaciones Científicas CSIC, Grupo de Ecología Genética de la Rizosfera, Granada, Spain

Backgrounds

High-throughput transcriptome profiling (RNAseq) has uncovered large and heterogeneous populations of small noncoding RNA species (sRNAs) with potential regulatory roles in bacteria. sRNAs mostly act as post-transcriptional regulators of gene expression to fine-tune cellular processes such as general responses to abiotic stress, quorum sensing, virulence or biofilm formation. The so-called trans-sRNAs regulate target mRNAs by protein-assisted short and discontinuous base-pairing interactions. Noncoding RNomics in rhizobia, the nitrogen-fixing symbionts of legumes, has been capitalized to date in the model alfalfa partner *Sinorhizobium meliloti.* The most informative data about trans-sRNA function is the identity of their target mRNAs. Because of imperfect base-pairing interactions, target identification requires experimental confirmation of *in silico* predictions.

Objectives

Development of a genetic reporter assay for the experimental validation of predicted sRNA-mRNA base-pairing interactions in *S. meliloti*.

Methods

Our assay is based on the co-transformation of bacteria with two compatible plasmids that independently overexpress the selected sRNAs and a translational fusion of the putative target mRNA interaction region to the reporter GFP. Here, we compared efficiency of sRNA constitutive and IPTG-inducible overexpression systems using known target mRNAs of the homologous AbcR1 and AbcR2 sRNAs, encoding ABC transporters.

Conclusions

Interestingly, we observed that sRNA inducible overexpression is more efficient than constitutive overexpression to verify sRNA-mRNA interactions. Using this method, we verified two new AbcR1 and AbcR2 targets, encoding periplasmic components of ABC transporters. One of them is also a new target of a yet uncharacterized sRNA influencing nodule formation efficiency (NfeR1).

FEMS7-1703
Physiology / Biochemistry / Molecular Microbiology

NONTYPABLE HAEMOPHILUS INFLUENZAE GENOMIC PATHO-ADAPTATION IN THE AIRWAYS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS WITH LONG-TERM MICROBIAL PERSISTENCE

J. Moleres[1], J.C. Mell[2], R. Ehrlich[2], F.C. Ariadna[1], S. Martí[3,4], S. Cuesta[1], L. Caballero[1], I. Rodriguez-Arce[1], G. Ehlrich[2], J. Liñares[3,4], J. Garmendia[1,4]
[1]Instituto de Agrobiotecnología, Animal Healthcare, Mutilva, Spain
[2]Drexel University, Microbiology & Immunology, Philadelphia, USA
[3]Hospital Universitario Bellvitge, Departamento de Microbiología, Barcelona, Spain
[4]Centro Investigación Biomédica en Red, Centro Investigación Biomédica en Red, Madrid, Spain

Backgrounds

The respiratory pathogen nontypeable *Haemophilus influenzae* (NTHi) is encountered in a significant proportion in the lower airways of Chronic Obstructive Pulmonary Disease (COPD) patients. The COPD lung is likely to be a unique niche favouring its adaptation to persistent infection.

Objectives

To unravel NTHi evolutionary dynamics in the lung of the COPD patient over time, and to identify microbial patho-adaptive features.

Methods

Ninety-two NTHi isolates from sputum samples of 13 COPD patients at exacerbation, recovered in a prospective longitudinal study (2000-2014), were genome sequenced: (a) Pacific Biosciences long-read sequencing was used to generate complete assemblies for one representative of each clonal type; (b) Illumina short-read sequencing was applied to all isolates. Genome assembly used a customized pipeline and raw reads were also aligned to the PacBio reference genomes to generate variant tables for each set of clonal type isolates. Functional characterization of parallel evolution-related allelic variation was analyzed for selected genes.

Conclusions

Ninety-two NTHi COPD isolates have been (i) phylogenomically analyzed in the context of all publicly available NTHi genome sequences, (ii) organized in 33 clonal types, (iii) used to establish variation in NTHi genome architecture, (iv) used to identify traits of parallel evolution by fixing at least one single nucleotide variant in more than one clonal type. Detailed analysis of the *fadL-ompP1* gene parallel evolution and allelic variation will be discussed, in terms of its role as an exogenous long-chain fatty acids transporter, as a ligand of the human receptor CEACAM-1 and as a source of antigenic variation.

FEMS7-2191
Physiology / Biochemistry / Molecular Microbiology

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF NEISSERIA GONORRHOEAE RESISTANT ISOLATES IN BUCHAREST, ROMANIA

V.C. Cristea[1], I. Gheorghe[2], A.I. Cotar[2], M. Popa[2], G. Gradisteanu[2], V. Burloiu[2], G.R. Pricop[1], V. Lazar[2], M.C. Chifiriuc[2]
[1]Central Reference Laboratory Synevo- Bucharest- Romania, Microbiology, Bucharest, Romania
[2]Faculty of Biology- University of Bucharest- Research Institute of the University of Bucharest, Microbiology, Bucharest, Romania

Backgrounds

Although gonporrhoea is among the top public health problems in Romania, leading to a series of complications, however, there is a lack of data regarding the antibiotic resistance of local *Neisseria gonorrhoeae* strains, while molecular typing methods have not yet been introduced for their molecular characterisation.

Objectives

The purpose was to characterize the *N. gonorrhoeae* recent isolates exhibiting antibiotic resistance to penicillin, tetracycline and fluoroquinolones using phenotypic methods, PCR and Antigen Sequence Typing (NG-MAST).

Methods

This study was conducted on 20 strains isolated from *January – June 2016* from men urethral secretions sent to Synevo Laboratory in Bucharest. Isolates were identified by mass spectrometry and antibiotic susceptibility was determined in accordance with the CLSI, 2016. PCR were performed on genomic DNA, in order to identify the penicillin resistance genes [TEM like β-lactamase; PenA and PonA (penicillin binding protein); mutation of the *mtrR* gene, outer membrane porin *PorB1b* and also the mutations in the *pilQ* gene], tetracycline resistance genes (*tetM* and mutation in the *rpsJ* gene) and fluoroquinolone resistance (mutations in *gyrA* and *parC*). The molecular study revealed the presence of the followed resistance genes: ciprofloxacin (*gyrA* – 45% and *parC* – 40%); tetracycline (*rpsJ* - 100% and tetM - 10%); penicillins (*PilQ* - 95%; *mtr* - 90%; *PonA* - 35%; *PenA* - 15*%; bla*$_{\text{TEMlike}}$ - 15% and *PorB* - 5%).

Conclusions

The molecular typing of resistant isolates may provide the capacity to identify possible associations between ST and AMR (antimicrobial resistance) phenotype and patient characteristics, having as the final purpose to guide treatment algorithms.

FEMS7-2264
Physiology / Biochemistry / Molecular Microbiology

THE SPREADING OF CTX-M-15 IN KLEBSIELLA PNEUMONIAE IS LINKED WITH MULTIDRUG RESISTANT PHENOTYPE

I. Czobor[1], I. Gheorghe[2], I. Avram[3], S. Petrut[3], V. Lazar[2], M.C. Chifiriuc[2], Z. Zhiyong[4]
[1]Faculty of Biology- University of Bucharest- Research Institute of the University of Bucharest, Genetics, Bucharest, Romania
[2]Faculty of Biology- University of Bucharest- Research Institute of the University of Bucharest, Microbiology, Bucharest, Romania
[3]Faculty of Biology- University of Bucharest, Genetics, Bucharest, Romania
[4]Centre of Infectious Diseases- West China Hospital- Sichuan University, Microbiology, Chengdu, China

Backgrounds

Acquired infections caused by multidrug-resistant CTX-M-15-producing *Klebsiella pneumoniae* have dramatically increased in recent years however, the extent of distribution in the country or the genetic support for their emergence and spread are unknown.

Objectives

The aim of this study was to analyze the population structure and genetic elements responsible for the dissemination of extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* identified in one main hospital in Bucharest, south of Romania.

Methods

Thirty-four *K. pneumoniae* isolates from different clinical specimens were selected (May 2016) according to multidrug resistant phenotype (MDR). Bacterial identification and antibiotic susceptibility testing were performed by standard methods. The presence of β–lactamases was investigated by PCR and sequencing and the population structure were investigated by Pulsed-field gel electrophoresis (PFGE) analysis. All *K. pneumoniae* strains were MDR, most frequently involving resistance to β-lactam antibiotics, aminoglycosides, quinolones and colistin. bla_{NDM-1} gene was detected only in one strain of *K. pneumoniae* whereas OXA-48 was identified in 25% of the analysed strains. One *K. pneumoniae* isolate produced both NDM and CTX-M, and 82% of the isolates produced CTX-M-15. 6% of isolates produced both CTX-M-15 and OXA-48 and another 6% co-produced TEM and OXA-48. Among ten PFGE-types identified, one of them was more frequently identified (n=15/34); none of the pulsotype could be linked to any specific resistance pattern.

Conclusions

CTX-M-15 is the most prevalent β-lactamase detected amongst the MDR *K. pneumoniae*. As there were observed a lot of pulsotypes, we can conclude that antibiotic resistance is not linked with a specific clone and it`s spreading in Romanian hospitals.

FEMS7-2979
Physiology / Biochemistry / Molecular Microbiology

STUDY INHIBITORY EFFECT OF PEPTIDE NUCLEIC ACIDS AGAINST FUSOBACTERIUM NUCLEATUM GROWTH BY TARGETED TO NECESSARY GENES

P. Gholizadeh[1], M. Aghazadeh[2], H. Samadi Kafil[3]
[1]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Tabriz University Of Medical Sciences, Medical Microbiology, Tabriz, Iran
[3]Drug Applied Research Center, Tabriz University Of Medical Sciences,Tabriz, Iran

Backgrounds

Fusobacterium nucleatum is an anaerobe, gram negative, fusiform or spindle shaped rods. It is associated with periodontal disease, gut disease (ulcerative colitis (UC), inflammatory bowel disease (IBD) and Crohn's disease) and it has an increased risk of oral cavity, pancreatic and colorectal cancers. Peptide nucleic acids (PNAs) are one of the DNA mimic, synthetic components that can bound to single strand and double strand DNA or RNA. Binding the peptide-PNA to PNA targeting DNA or RNA inhibit expression or translation of PNA targeting genes.

Objectives

We are developing PNA targeted to *16srRNA*, *rpoD* (encoded RNA polymerase subunit sigma) and *gyrA* (encoded DNA gyrase subunit A) genes in *F. nucleatum* isolates.

Methods

Peptide-PNAs targeted to conserve sequence of *16srRNA*, *rpoD* and *gyrA* genes in *F. nucleatum* isolated from biopsy samples of colorectal cancer patients in vitro.

Conclusions

The initiate results indicated that PPNAs could inhibit expression of *16srRNA*, *rpoD* and *gyrA* and it seems to be successful effect on inhibition of *F. nucleatum* isolates growth. However, based on other results that evaluated PPNA targeting necessary genes effects on *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus*, it may demonstrate that conserve sequence of *16srRNA*, *rpoD* and *gyrA* are possible targets for *F. nucleatum*. Finally, PPNAs provide novel sequence designed and ultra-spectrum antibiotics based on DNA antisense and antigene strategies.

FEMS7-2980
Physiology / Biochemistry / Molecular Microbiology

CRSIPR/CAS SYSTEM EFFECTS ON ACQUIRED DRUG RESISTANCE AND VIRULENCE FACTORS OF STAPHYLOCOCCUS AUREUS

P. Gholizadeh[1], M. Aghazadeh[2], H. Samadi Kafil[3]
[1]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran(2)Tabriz university of medical sciences, Department of medical microbiology, Tabriz, Iran
[3]Drug Applied Research Center, Tabriz University Of Medical Sciences,Tabriz, Iran

Backgrounds

CRSIPR/Cas systems are adaptive immune system of eubacteria and archaebacteria that called clustered regularity interspaced short palindromic repeats coupled with CRISPR-associated (Cas) proteins. These systems provide adaptive immunity against potentiality dangerous foreign DNA for example bacteriophages DNA, plasmids and transposable elements. *Staphylococcus aureus* is common multi-drug resistant that causes community and hospital acquired infections. Many of the antibiotic resistant phenotypes of these organisms have been carried by R-plasmids that CRISPR/Cas system may control these plasmids.

Objectives

Recent studies demonstrated that CRISPR/Cas system can control and manage VRSA and MRSA plasmids in *S. aureus*.

Methods

We tend to searched CRISPR/Cas system, *S. aureus*, and antibiotic resistance and relevance of full texts on papers were studied in the roles of CRISPR/Cas in VRSA and MRSA plasmids.

Conclusions

The *nickase* gene is homologous with spacer 1 (*spc1*) locus in CRISPR loci and presented in conjugation plasmids of vancomycin resistant and methicillin resistant *S. aureus* strains. The results showed its efficacy on self-splicing intron insertion into MRSA and VRSA plasmids and it interferes with target plasmid directly. If CRISPR/Cas system can be manipulated in clinical treat, these system would provide limitation and also inhibition of spread of antibiotic resistance plasmid genes such as VRSA and MRSA. Development these system may resolve the acquisition of antibiotic resistance and virulence factor problems in potentiality pathogens and open novel vision for development therapeutic strategies.

FEMS7-0032
Physiology / Biochemistry / Molecular Microbiology

SUBSTITUTION OF ALANINE AT POSITION 184 IN Ω -LIKE LOOP WITH GLUTAMIC ACID INTRODUCES BETA-LACTAMASE ACTIVITY IN ESCHERICHIA COLI PBP5

A. Ghosh[1], *D. Kar*[1], *S.D. Pandey*[1], *S. Mallick*[1], *M. Dutta*[1]
[1]*Indian Institute of Technology Kharagpur, Biotechnology, Kharagpur, India*

Backgrounds

E. coli PBP5 is a DD-carboxypeptidase that helps in maintaining cell shape and intrinsic beta-lactam resistance. Though, PBP5 does not have beta-lactamase activity under physiological pH, structurally it shares a common but a shorter Ω-like loop, resembling class A beta-lactamases. However, the key glutamic acid residue present in the Ω-loop of beta-lactamase is absent in the Ω-like loop of PBP5. It is assumed that beta-lactamases and DD-carboxypeptidases may undergo divergent evolution leading to distinct functional enzymes with different substrate specificities indicating the versatility of Ω-loops, though supporting experimental evidence is insufficient.

Objectives

To investigate the physiological and biochemical effects of introducing glutamic acid in PBP5 Ω-like loop.

Methods

We substituted A184 to E to create PBP5_A184E. Ectopic expression of PBP5_A184E in *E. coli* ΔPBP5 mutant markedly elevates the beta-lactam resistance, especially towards cephalosporin. However, like PBP5, PBP5_A184E retains the ability to complement the aberrantly shaped *E. coli* septuple PBP mutant indicating unaltered *in vivo* DD-carboxypeptidase activity. Enzyme kinetics of the purified soluble PBP5 and PBP5_A184E, and bioinformatics analyses have substantiated the dual enzyme nature of A184E mutated protein possessing both DD-carboxypeptidase and beta-lactamase activities. Apart from to introducing favorable conditions for beta-lactam hydrolysis, the groove volume of active-site has also been increased by ~20%, which is similar to the enhanced groove volume observed in the dual enzyme of *M. smegmatis*.

Conclusions

Therefore, substitution of A184 to E at Ω-like loop alone can introduce a beta-lactamase activity in *E. coli* PBP5 without hampering its DD-carboxypeptidase activity supporting the possibility of single amino acid polymorphism.

FEMS7-0439
Physiology / Biochemistry / Molecular Microbiology

BIOCHEMICAL CHARACTERIZATION OF A VARIANT OF ESCHERICHIA COLI GLUTAMATE DECARBOXYLASE WITH IMPROVED GABA PRODUCTION AT ALKALINE PH

F. Giovannercole[1], E. Pennacchietti[1], D. De Biase[1]
[1]Sapienza University of Rome, Department of Medical-Surgical Sciences and Biotechnologies- Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Latina, Italy

Backgrounds

Glutamate decarboxylase (Gad), a structural component of the major acid resistance system in many orally-acquired, neutralophilic bacteria, yields CO_2 and γ-aminobutyric acid (GABA), while consuming one proton/catalytic cycle [1]. *Escherichia coli* GadB (*Ec*GadB) has a pH optimum in the pH range 4-5 and displays no activity at pH ≥ 6.5. Intracellularly, the enzyme activity is therefore pH-controlled. Based on the available crystal structures of *Ec*GadB, residues Asp86 and His465, highly conserved in bacterial Gads, were suggested to be major players in the control of *Ec*GadB activity [1].

Objectives

Bacterial Gad is regarded as an interesting tool for “green chemistry” applications because it can be employed to synthesize GABA, a non-proteinaceous amino acid, highly desirable in functional food and precursor of 2-pyrrolidone (an industrial solvent) for the synthesis of nylon 4 [2]. We engineered *Ec*GadB at the level of residues Asp86 and His465 to generate a variant less sensitive to pH increase.

Methods

*Ec*GadB_Asp86Asn-His465Ala was overexpressed and purified. UV-visible and fluorescence spectrophotometry, circular dichroism, pH-dependent enzymatic activity assays and solvent kinetic isotope effect were employed to characterize this variant.

Conclusions

*Ec*GadB_Asp86Asn-His465Ala, while retaining substrate specificity, acts as a robust catalyst in the pH range 7-8 and displays an altered solvent isotope effect. Because pH is no longer a limiting reaction parameter for the mutant enzyme, we suggest that this variant can be a better tool than wild type *Ec*GadB for the industrial synthesis of GABA.

References

[1] De Biase D, Pennacchietti E. (2012) *Mol. Microbiol* **86**: 770-86.
[2] Lammens TM et al. (2009) *Green Chemistry* **11**: 1562-67.

FEMS7-2723
Physiology / Biochemistry / Molecular Microbiology

PIVOTAL ROLE OF MRNA STABILITY REGULATION IN E. COLI ADAPTATION

M. Cocaign-bousquet[1], S. Nouaille[2], S. Laguerre[3], B. Enjalbert[1], L. Girbal[1]
[1]LISBP, CNRS INRA INSA, toulouse, France
[2]LISBP, INRA CNRS INSA, Toulouse, France
[3]LISBP, INSA INRA CNRS, Toulouse, France

Backgrounds

Understanding bacterial adaptation is essential to master bacterial proliferation and capacities and is a prerequisite for its efficient use in biotechnology. Bacteria constantly adapt their growth and metabolism to changing environments through complex and partially understood regulatory networks.

Objectives

A vast reorganisation at the transcriptional level coordinated by global and/or specific regulations is known to occur. However how this transcriptional reorganization progress through other intracellular levels remains unclear and the importance of post-transcriptional regulation is undetermined. We have investigated here how the post-transcriptional regulations of mRNA stability impact adaptation of the model bacterium *E. coli*.

Methods

We have measured stabilome (individual mRNA half-life for all bacterial transcripts) in various environments (Esquerre et al., NAR 2014) to evaluate if mRNA stability regulations influence mRNA dynamics. From these Omics data we have computed regulation coefficients and analysed the coordination of mRNA stability control with transcription regulation. Systemic analysis was performed to identify the major determinants of mRNA stability (Esquerre et al. BMC genomics 2015) and to investigate particular regulations. The post-transcriptional CSR system was demonstrated to control thousands of mRNA stabilities impacting significantly the central carbon metabolism and bacterial physiology (Esquerre et al. Sci Rep. 2016, Morin et al. Mol Microbiol 2016). Lastly, we have demonstrated by *in vivo* kinetics approaches that global regulations of mRNA stability do exist in bacteria and superimposed to specific regulations.

Conclusions

mRNA stability is a sensitive process influencing significantly the mRNA dynamics with important consequences in bacterial physiology. This work demonstrates the pivotal role of post-transcriptional regulations.

FEMS7-1284
Physiology / Biochemistry / Molecular Microbiology

ANALYSIS OF INTRACELLULAR NADPH ACCUMULATION KINETICS IN C. GLUTAMICUM USING THE GENETICALLY ENCODED SENSOR-PROBE MBFP_CG

O. Goldbeck[1], *G. Seibold*[1]
[1]*Institute of Microbiology and Biotechnology, AG Eikmanns, Ulm, Germany*

Backgrounds

The Gram-positive *Corynebacterium glutamicum* is used in biotechnological applications which come along with high demands for intracellular NADPH. So far, these demands were mainly covered by genetic engineering of the pentose phosphate pathway. However, strains that excessively use the PPP often suffer from negative side-effects. The redirection of the carbon-flux toward the PPP by using a phosphoglucoisomerase-negative strain led to an improvement of *C. glutamicum,* but was accompanied by negative effects on growth and sugar uptake.

Objectives

Inhibition of sugar uptake occurred in *C. glutamicum* Δ*pgi* within seconds, which might be triggered by high intracellular NADPH concentrations. However, kinetics of NADPH accumulation have not been investigated in bacteria so far, due to the lake of tools for fast online-analysis. The sensor probe mBFP_Cg was created to solve this problem.

Methods

The protein mBFP was used for *in vitro* and *in vivo* assays. The protein was biochemically characterized and optimized using genesynthesis for its application in *C. glutamicum*, resulting in the protein mBFP_Cg. The plasmid pMBFP_Cg was used as a genetically encoded sensor to analyze kinetics of NADPH accumulation in *C. glutamicum* strains with altered NADPH metabolism.

Conclusions

Fast accumulation was observed in *C. glutamicum* Δ*pgi*. Strains expressing genes for transhydrogenases were checked upon their NADPH accumulation behavior. A method was established to determine the kinetic background of sugar uptake inhibition in *C. glutamicum* Δ*pgi*. The addition of glucose leads to an accumulation of NADPH resulting in the inhibition of the Glucose-6-phosphate-dehydrogenase. Following this event, Glucose-6-Phosphate accumulates and leads to the inhibition of sugar-transporters.

FEMS7-1133
Physiology / Biochemistry / Molecular Microbiology

DIFFERENTIAL REGULATION OF ACTIVATION AND STABILIZATION OF PKC ORTHOLOGS IN FISSION YEAST

E. Gómez Gil[1], B. Vázquez-Marín[1], T. Soto[1], A. Franco[1], J. Vicente-Soler[1], M. Gacto[1], P. Pérez[2], J. Cansado[1], M. Madrid[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain
[2]Instituo de Biología Funcional y Genómica IBFG- Consejo Superior de Investigaciones Científicas, Morphogenesis and cell polarity, Salamanca, Spain

Backgrounds

The two Protein kinase C orthologs Pck1 and Pck2 operate in a redundant fashion in the fission yeast *Schizosaccharomyces pombe* to control essential functions including morphogenesis, cell wall biosynthesis, and the activity of the cell integrity pathway (CIP) and is core element MAP kinase Pmk1.

Objectives

To identify the mechanisms responsible for maturation, catalytic activation, and stabilization of Pck1 and Pck2.

Methods

We show that despite their strong structural similarity and functional redundancy, the mechanisms regulating their maturation, activation, and stabilization have a remarkably distinct biological impact on both kinases. Contrary to Pck2, putative *in vivo* phosphorylation of Pck1 within conserved activation loop, turn and hydrophobic motifs is essential for protein stability and biological functions. Constitutive activation promoted dephosphorylation and destabilization of Pck2, while it enhanced Pck1 stability to interfere with proper downstream signaling to the CIP triggered by Pck2. Importantly, whereas catalytic activity is essential for Pck1 functions, Pck2 remains partially functional in absence of kinase activity.

Conclusions

Our findings suggest that early duplication from a single ancestor involved important changes in the mechanisms regulating catalytic activation and stability of PKC family members to allow for a flexible and dynamic control of downstream functions, including MAPK signaling.

FEMS7-1678
Physiology / Biochemistry / Molecular Microbiology

THE CHROMOSOME ARCHITECTURE OF STREPTOCOCCUS PNEUMONIAE IS OPTIMIZED FOR PHYSIOLOGY AND PATHOGENICITY

A. González de la Campa[1], *A.J. Martín-Galiano*[1], *F. Maria José*[1]
[1]*Centro Nacional de Microbiología. Instituto de Salud Carlos III, Unidad de Genética Bacteriana, Majadahonda, Spain*

Backgrounds

The transcriptome of *Streptococcus pneumoniae* is affected by changes in DNA-supercoiling. Relaxation of DNA with the gyrase inhibitor novobiocin reveals than 36% of the genome is differentially-expressed, these genes forming 15 topology-reacting domains. These domains are categorized as up-regulated (UP), down-regulated (DOWN), non-regulated (NR), and AT-rich.

Objectives

To study the characteristics of the diverse domains and the functionalities of their genes

Methods

Location Dispersion Index, Codon adaptation index, Protein-protein interactions

Conclusions

Genes of NR domains were subcategorized into position-conserved non-regulated (pcNR, 17% of the genome) and position-variable non-regulated (pvNR, 36% of the genome) domains. On average, pcNR domains showed high transcription rates, optimized codon usage, and were found to contain a small number of RUP/BOX/SPLICE repeats. These domains were enriched in leading strand genes, essential genes and those that code for proteins involved in primary metabolism but poor in exogenous genes. In contrast, pvNR genes coding for cell wall proteins, paralogs, virulence factors and immunogenic candidates for protein-based vaccines were found to be overrepresented. DOWN domains were enriched in genes essential for infection. Many UP and DOWN domain genes were activated during different stages of competence, whereas pcNR genes tended to be repressed until the competence was switched off. Pneumococcal genes appear to be subject to a topology-driven selection pressure that defines the chromosomal location of genes involved in metabolism, virulence and competence. The pcNR domains are interleaved between UP and DOWN domains according to a pattern that suggests the existence of macrodomain entities of around 200 Kb.

FEMS7-1772
Physiology / Biochemistry / Molecular Microbiology

ROLE OF TMRNA IN ANTIBIOTIC SUSCEPTIBILITY IN STREPTOCOCCUS PNEUMONIAE

A. González de la Campa[1], L. Brito[2], J. Wilton[2], M.J. Ferrándiz[1], A. Gómez-Sanz[2], M. Amblar[2]
[1]Centro Nacional de Microbiología. Instituto de Salud Carlos III, Unidad de Genética Bacteriana, Majadahonda, Spain
[2]Centro Nacional de Microbiología. Instituto de Salud Carlos III,
Unidad de Patología Molecular del Neumococo, Majadahonda, Spain

Backgrounds

The tmRNA, encoded by the *ssrA* gene, is a small non-coding RNA involved in *trans*-translation that contributes to the recycling of ribosomes stalled on aberrant mRNAs. In most bacteria, its inactivation has been related with a decreased ability to respond to and recover from a variety of stress conditions.

Objectives

To investigate the role of tmRNA and trans-translation under several stresses, including antibiotic stress, in the human pathogen *Streptococcus pneumoniae*

Methods

Survival rates, construction of genetically-modified strains, efflux, reactive oxygen species (ROS) detection, chromosome fragmentation

Conclusions

A D*ssrA* strain grew slower than the wild type, indicating that is not essential in the pneumococcus. This strain was more sensitive to UV irradiation, exogenous H_2O_2, and antibiotics inhibiting translation and transcription. Remarkably, D*ssrA* was more resistant to fluoroquinolones, showing higher MIC values and survival rates. However, the D*ssrA* strain showed higher accumulation of ethidium bromide and levofloxacin than the wild type. Production of ROS and chromosome fragmentation associated to moxifloxacin and levofloxacin were reduced. Moreover, such protective effect relay mainly on inhibition of protein synthesis, since similar protection against levofloxacin was observed under previous treatment with antibiotics inhibiting translation. Therefore tmRNA has a protective effect under several types of stresses. However, upon FQ treatment, tmRNA is paradoxically harmful. These findings should be taking in consideration in the development of new antibiotics inhibiting trans-translation. In addition, although not currently used in clinical practice, our results suggest that the combined use of FQs and antibiotics inhibiting protein synthesis might not be recommended.

FEMS7-1240
Physiology / Biochemistry / Molecular Microbiology

PTC1, A SERIN/THREONIN PHOSPHATASE REGULATING MAPKS PATHWAYS AND MORPHOGENETIC PROCESSES IN SACCHAROMYCES CEREVISIAE THROUGH ITS ADAPTOR PROTEIN NBP2

G. González-Rubio[1], A. Sacristán-Reviriego[1], L. Palacios[1], H. Martín[1], M. Molina[1]
[1]Complutense University of Madrid. School of Pharmacy, Department of Microbiology II, Madrid, Spain

Backgrounds

The type 2C protein phosphatase Ptc1 is the closest yeast homolog of human Wip1, which has been reported to be involved in the regulation of oncogenic processes. It has been shown that Ptc1, together with its protein adaptor Nbp2, has an essential role in the negative regulation of both the Hog1-mediated High Osmolarity (HOG) and the Slt2-mediated cell wall integrity (CWI) MAPK pathways. Loss of Ptc1 leads to numerous phenotypic defects including cell wall stress sensitivity and the delay of organelles inheritance in *S. cerevisiae* cells. Moreover, *ptc1* mutants displayed a multibudded phenotype when grown at 37ºC, Nevertheless, many of the mechanisms underlying these phenotypic defects remain unknown.

Objectives

The goal of this study was to gain insight into the morphogenetic defects that have been observed in Ptc1-deficient cells as well as to know if the absence of its protein adaptor, Nbp2, leads to the same effects.

Methods

In this work we characterized different mutants of *S. cerevisiae* employing the next techniques: gene expression studies, Western blotting analyses and fluorescence and Differential Interference Contrast (DIC) Microscopy.

Conclusions

Lack of Ptc1 protein adaptor Nbp2 results in the hyperactivation of HOG and CWI pathways, and the same multibudding phenotype at 37ºC as the *ptc1*Δ mutant. In both mutants, every bud contains a nucleus, but a still assembled septin ring, in contrast to that observed in the mother cell side. This phenomenon provides an excellent experimental setting to gain insight into the molecular mechanism connecting cell separation and septin disassembly.

FEMS7-1448
Physiology / Biochemistry / Molecular Microbiology

MOLECULAR TIPYNG OF CLOSTRIDIUM DIFFICILE ISOLATES FROM PATIENTS IN THREE HOSPITALS OF HIGH COMPLEXITY OF MEDELLIN, COLOMBIA

C.L. Salazar[1], C. Reyes[1], S. Atehortua[2], P. Sierra[3], M. Correa[1], W.N. Fawley[4], A. Gonzalez[1]
[1]Universidad de Antioquia, Microbiology School, Medellin, Colombia
[2]Hospital Universitario San Vicente Fundación, Microbiology, Medellin, Colombia
[3]Clínica León XIII, IPS UNiversitaria, Medellin, Colombia
[4]Leeds Teaching Hospitals NHS Trust, Departament of Microbiology, Leeds, United Kingdom

Backgrounds

Hypervirulent genotypes of *Clostridium difficile* have frequently been associated to hospital outbreaks of diarrhea in Europe and North America during the last two decades. However, in Colombia, genotypes of this bacterium have not yet been described.

Objectives

To molecularly characterize clinical isolates of *C. difficile* obtained from patients with suspicion of *C. difficile* associated disease (CDAD).

Methods

Stool samples were analyzed to determine the presence of A/B toxins using EIA. *C. difficile* was isolated by culture. DNA was extracted and PCR applied to detect *tcdA*, *tcdB* and binary toxin (CDTa/CDTb) genes. PCR-ribotyping was performed using a sequencer based on capillary electrophoresis (ABI-3100).

Conclusions

A total of 913 stool samples collected during 2013-2014 were evaluated. A total of 143 isolates of *C. difficile* were recovered from culture. The frequency of toxins A/B was 9.3% (85/913).After amplification, 100 (70%) isolates were *tcdA*+/*tcdB*+, 11 (7.8%) *tcdA*-/*tcdB*+ and 32 (22.4%) *tcdA*-/*tcdB*-. The most frequent ribotype was 591 (20%), followed by 106 (9%) and 002 (7.9%); only one isolate corresponded to ribotype 027 (considered hypervirulent); four ribotype 078 isolates and four new ribotypes (794,795, 804,805) were identified.

A low frequency of CDAD was detected in comparison to other countries. The *tcdA*+/*tcdB*+-CDTa/CDTb+ gene profile was correlated with epidemic ribotypes. There was a low frequency of ribotype 027 which is common in other countries. Interestingly, a high frequency of ribotype 591,of low circulation in Europe, was observed, followed by ribotypes 106 and 002, all of them considered epidemic in Europe dufingn the past decade.

FEMS7-2052
Physiology / Biochemistry / Molecular Microbiology

QUORUM SENSING BASED ON INTERSPECIES COMMUNICATION AMONG E. COLI SE15 AND OTHER STRAINS OF BIOFLM IN THE PATIENTS INDWELLING URINARY CATHETERS: GENOME EDITING OF CRISPR-CAS9

S.R. Kang[1], J. Kim[2], J.K. Hur[3], S.S. Lee[1]
[1]Kyonggi University, Department of Bio-engineering, Suwon, Republic of Korea
[2]Kyonggi University, Department of Life Science, Suwon, Republic of Korea
[3]Korea University, Biomedical Research Center- Anam Hospital, Seoul, Republic of Korea

Backgrounds

The predominant strains *E. coli*, *P. aeruginosa* and *S. aureus* are the most common pathogens in catheter associated urinary tract infections (CA UTIs). The CA UTIs were mainly occurred from biofilm formation by Quorum Sensing (QS) system of predominant strains. Moreover, AI-2, one of QS signal molecules, plays an important role in enabling interspecies communication. Although many bacteria species have been studied to use the AI-2 for interspecies communication to regulate various behaviors, such as biofilm formation, the molecular mechanisms of AI-2 internalization and signal transduction remain poorly understood.

Objectives

This study is to elucidate interaction between *E. coli* SE15 and other dominant strains, which frequently form biofilm on the catheter surface, via co-culture.

Methods

We then manipulated the Δ*rbsB* and Δ*luxS* in *E. coli* SE15 by using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system. And we observed biofilm formation assay in Δ*rbsB* and Δ*luxS* mutants of *E. coli* SE15. Moreover, Δ*rbsB* and Δ*luxS* mutants of *E. coli* SE15 were co-cultured with predominant strains *P. aeruginosa* and *S. aureus* in response to the wild type *E. coli* and analyzed AI-related genes expression by qRT-PCR.

Conclusions

Autoinducer synthesis gene *luxS* gene of *E. coli* SE15 has been affected by other dominant strains because the gene expression was more increased and rapidly initiated in co-culture than single culture. Based on these data, we concluded that *luxS* and *rbsB* affected on interaction of other predominant bacteria. Such interactions among predominant bacteria were helpful to understand their symbiosis or competition.

FEMS7-0384
Physiology / Biochemistry / Molecular Microbiology

METAGENOMIC CHARACTERIZATION OF THE SOS TRANSCRIPTIONAL RESPONSE IN THE PATESCIBACTERIA SUPERPHYLUM

M. Sánchez-Osuna[1], *J. Barbé*[1], *I. Erill*[2]
[1]*Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, Bellaterra, Spain*
[2]*University of Maryland- Baltimore County, Department of Biological Sciences, Baltimore, USA*

Backgrounds

Metagenomics and single-cell genomics provide unprecedented insights into the genetic composition of microbial communities inhabiting natural environments. Recent surveys have uncovered a major radiation of candidate phyla in the Bacteria domain, represented predominantly by the proposed Patescibacteria superphylum. Patescibacteria have small genomes (<1Mbp) and a limited genetic repertoire that suggest a symbiotic or parasitic lifestyle.

Objectives

In order to shed light on the lifestyle and evolution of these uncultivated organisms we combine *in silico* and *in vitro* approaches to define LexA-binding motif of the Patescibacteria, and we leverage metagenomics data to characterize the SOS regulatory network in this superphylum.

Methods

Orthologs for Patescibacteria LexA were identified through reciprocal BLASTP and their upstream sequences were submitted to MEME to perform motif discovery. Having identified the LexA-binding motif, we searched for instances of it in Patescibacteria whole genome shotgun assemblies and comparative genomics analyses were performed in order to reconstruct the putative SOS regulon. EMSA assays validated and defined the computationally predicted LexA-binding sites.

Conclusions

Our work shows that the Patescibacteria LexA controls a small core set of SOS genes by binding to a novel LexA-binding motif with an unusual direct-repeat structure, complemented by varying degrees of LexA regulation of other SOS functions. These results suggest that the presence of a functional SOS response hints an intermediate step in the process of bacterial genomic reduction resulting from intimate association with a host species or a structured community, and may thus provide insights into the lifestyle of uncultivated bacteria.

FEMS7-1753
Physiology / Biochemistry / Molecular Microbiology - Part II

THE MOLECULAR BASIS OF THE PLANT GROWTH PROMOTING CAPACITY OF STREPTOMYCES SP.

S. Goormachtig[1], S. Langendries[2], A. Lampens[2], T. Viaene[2]
[1]Ghent University - VIB, Plant Systems Biology, Ghent, Belgium
[2]Ghent University - VIB- Plant Systems Biology- Ghent- Belgium., Plant Systems Biology, Ghent, Belgium

Backgrounds

Streptomyces are described as powerful plant growth promoting rhizobacteria which contribute significantly to the composition of the root microbiome in the model plant *A. thaliana* as well as important crops such as rice and maize. Although Streptomyces strains have been shown to produce siderophores, volatile organic compounds and plant hormones like auxin and cytokinin and elicit induced systemic resistance, more investigations are required to understand the mechanisms by which these PGPR strains are perceived by the plants and evoke growth promotion and/or plant protection.

Objectives

We have identified several Streptomyces strains that promote growth of Arabidopsis as well as important crops such as wheat and maize. We aim at understanding the plant signaling networks on which the bacteria impinge to provoke the growth promoting traits.

Methods

We have analyzed the phenotypic effects provoked by different streptomyces sp., through the analysis of Arabidopsis mutants and marker lines.

Conclusions

Our study revealed two different mechanisms by which Streptomyces strains promote growth in Arabidopsis, one by impinging on the jasmonate response while a second one impinging on the cytokinin response.

FEMS7-1182
Physiology / Biochemistry / Molecular Microbiology - Part II

A NEW MODEL FOR BACTERIAL PERSISTENCE TO OFLOXACIN

F. Goormaghtigh[1], *L. Van Melderen*[1]
[1]*Free University of Brussels ULB, Département de Biologie Moléculaire, Gosselies, Belgium*

Backgrounds

Together with the current antibiotic resistance crisis, bacterial persistence appears to play an increasingly important role in the frequent failure of antibiotic treatments. Persister cells are rare bacteria that transiently become drug tolerant, allowing them to survive lethal concentrations of antibiotics. The specific physiology of persisters has been shown to rely on several global molecular mechanisms, such as the SOS response, the stringent response and/or dormancy. As a result, development of efficient anti-persister treatments remains elusive.

Objectives

We aim at a better understanding of the specific molecular mechanisms leading to persistence to ofloxacin in *E. coli* and allowing persisters to elude the lethal effect of ofloxacin, a question which has mostly been overlooked previously.

Methods

A mathematical model of the ofloxacin killing curve was developed and metabolic parameters were measured in the culture. Persisters were analysed at the single-cell level, in a microfluidic device. Finally, populational and single-cell analyses were combined to develop a working model for persistence to ofloxacin.

Conclusions

We showed that on the contrary to most antibiotics, ofloxacin treatment does not yield a biphasic killing curve, but a complex killing curve composed of four different phases. We confirmed the major role of the SOS response both at the population and at the single-cell level. Interestingly, using a microfluidic device, we observed that the SOS response only allows cells to recover more efficiently after ofloxacin removal. Finally, we showed that addition of a chemical inhibitor of the SOS response appears to be a promising strategy to eradicate ofloxacin persisters.

FEMS7-1630
Physiology / Biochemistry / Molecular Microbiology - Part II

METABOLIC PARAMETERS INFLUENCING PERSISTENCE

F. Goormaghtigh[1], *L. Van Melderen*[1]
[1]*Free University of Brussels ULB, Département de Biologie Moléculaire, Gosselies, Belgium*

Backgrounds

Together with the current antibiotic resistance crisis, bacterial persistence appears to play an increasingly important role in the frequent failure of antibiotic treatments. Persister cells are rare bacteria that transiently become drug tolerant, allowing them to survive lethal concentrations of antibiotics. Although interest in the persistence phenomenon seriously increased in the past decade, persister cells physiology remains elusive up to now.

Objectives

We aim at characterizing persister cells metabolism and establishing quantitative relationships between persistence and metabolic activities such as DNA replication, growth rate, induction of efflux pumps and induction of stress responses.

Methods

A mathematical model was used to describe persisters time-kill curves. A combination of genetic, chemical and microscopic analyses was performed to characterize the physiological nature of *E. coli* persisters to ampicillin and ofloxacin.

Conclusions

We first showed that while ampicillin treatment yields a typical biphasic killing curve, ofloxacin treatment results in a very complex killing curve composed of four different phases. One of these phases is a regrowth phase, which is likely to rely on an increased plating efficiency rather than bacterial divisions in the presence of the antibiotic. We then confirmed the direct link between persistence and the growth rate using chemostat experiments. Further analyses revealed this link to be correlated with the ppGpp production observed at early treatment times and independent of DNA synthesis rate.

FEMS7-2126
Physiology / Biochemistry / Molecular Microbiology - Part II

OVEREXPRESSION OF THE PLEIOTROPIC REGULATOR CODY DECREASES SPORULATION, ATTACHMENT AND PELLICLE FORMATION IN BACILLUS ANTHRACIS

M. Gopalani[1], *R. Bhatnagar*[1]
[1]*Jawaharlal Nehru University, School of Biotechnology, New Delhi, India*

Backgrounds

CodY is a pleiotropic transcriptional regulator conserved in many low G+C gram-positive bacteria, where it actuates adaptation in response to the nutritional and energetic status of the cell. CodY senses the intracellular GTP and Branched-chain amino acids (BCAAs) levels and brings about appropriate changes in the transcriptome of the cell. In *Bacillus anthracis,* the top rated bioterrorism agent, CodY regulates around 500 genes and is required for the attainment of full virulence by the pathogen. However, its implication in the physiology of the pathogen like sporulation, biofilm formation etc is yet unexplored.

Objectives

Our study focuses on the regulation of CodY itself in different growth phases and nutrient deprived conditions in *B. anthracis*. We also determined its connotation in the physiology of *B. anthracis.*

Methods

qRT-PCR, immunoblotting, electroporation, titer estimation.

Conclusions

The cellular levels of CodY during the exponential to stationary phase transition and under different nutrient limiting conditions remained constant in *B. anthracis*. Immunoblotting studies revealed the presence of CodY in the whole spore lysate and secretome of *B. anthracis*. Further, CodY was overexpressed in *B. anthracis* Sterne strain by electroporation which led to a 100-fold decrease in the sporulation titer and a 2.5-fold decrease in the *in vitro* attachment ability of the bacteria. The pellicle formation by overexpressed strain was substantially low as compared to wild type bacilli. The overexpressed strain exhibited chaining phenotype during growth in liquid media and pellicle. Thus, this study provides insights into the role of this regulator in sporulation, pellicle and biofilm formation of *B. anthracis.*

FEMS7-1157
Physiology / Biochemistry / Molecular Microbiology - Part II

PECTOBACTERIUM ATROSEPTICUM EXOPOLYSACCHARIDES: IDENTIFICATION, MOLECULAR STRUCTURE, AND CONTRIBUTION TO BACTERIAL VIRULENCE

V. Gorshkov[1,2], *B. Islamov*[1], *P. Mikshina*[3], *G. Burygin*[4], *A. Shashkov*[5], *O. Petrova*[1], *V. Vorob'ev*[1,6], *Y. Gogolev*[1,2]
[1]*Kazan Institute of Biochemistry and Biophysics - RAS, Molecular Biology, Kazan, Russia*
[2]*Kazan Federal University, Biochemistry and Biotechnology, Kazan, Russia*
[3]*Kazan Institute of Biochemistry and Biophysics - RAS, Mecchanisms of Plant Cell Growth, Kazan, Russia*
[4]*Institute of Biochemistry and Physiology of Plants and Microorganisms - RAS, Immunochemistry, Saratov, Russia*
[5]*Zelinsky Institute of Organic Chemistry - RAS, Structural Research, Moscow, Russia*
[6]*Kazan Federal University, Botany and Plant Physiology, Kazan, Russia*

Backgrounds

Bacterial exopolysaccharides (EPS) are structurally and functionally diverse polymers that perform many biological functions and confer multiple properties to microbial population, in particular providing the formation of biofilms. *Pectobacterium atrosepticum* (*Pba*) – a causative agent of plant rots all over the world, was demonstrated to form biofilm-like structures – bacterial emboli *in planta* that is necessary for effective systemic plant colonization. Herewith, neither the role of *Pba* EPS was demonstrated in the formation of bacterial emboli nor these polymers were yet described for pectobacteria.

Objectives

In the present study, we attempted to identify and characterize the molecular structure of *Pba* EPS and to assess their role in plant colonization and formation of bacterial emboli in particular.

Methods

Pba EPS were characterized by various methods of chromatography, NMR spectroscopy and dynamic light scattering. For immunodetection of *Pba* EPS, specific antibodies were obtained. Various models were applied to analyze contribution of *Pba* EPS to plant colonization.

Conclusions

Pba was shown to synthesize polydisperse EPS (MW 100-800 kDa) with a backbone consisted of [→3)-α-D-Gal*p*-(1→2)-α-D-Man*p*-(1→4)-α-L-Rha*p*-(1→] and the side chains of β-D-erwiniose-(1→3)-α-D-Gal*p*-(1→ attached to mannopyranosyl residue at O-3 position. The synthesis of EPS was increased during stress conditions and especially during host plant colonization. Immunolabeling showed that *Pba* EPS were included in the extracellular matrix of bacterial emboli. Characterization of rheological and phytoimmune properties of these polymers pointed to their significant role in plant-pectobacteria interactions. This study was supported by RSF (15-14-10022) and a grant MK-2191.2017.4.

FEMS7-2261
Physiology / Biochemistry / Molecular Microbiology - Part II

HORIZONTAL TRANSMISSION FACTORS SPREADING ANTIBIOTIC RESISTANCE

F. Graf[1,2,3], H. Alalam[1,2,3], M. Zackrisson[1], P. Sunnerhagen[1,2], J. Warringer[1,2], A. Farewell[1,2]
[1]University of Gothenburg, Department of Chemistry and Molecular Biology, Gothenburg, Sweden
[2]University of Gothenburg, Centre for Antibiotic Resistance Research CARe, Gothenburg, Sweden
[3´], coauthor, contributed equally, Sweden

Backgrounds

Horizontal gene transfer accelerates the spread of antibiotic resistance genes among bacteria within and between infected hosts, and likely also in the environment. Conjugation of plasmids is thought to be the predominant form of spreading (multi-)drug resistance through horizontal gene transfer. The chromosomal factors controlling the rate of antibiotics resistance transmission by conjugation, however, remain largely unknown.

Objectives

We aim to identify those genetic host factors with the ultimate aim to develop drugs that inhibit conjugation and thereby the spread of antibiotic resistance genes to use in combination therapy with antibiotics.

Methods

We developed a massively parallelized system to accurately monitor conjugation of resistance plasmids in real time in *E. coli*. We screened the complete collection of *E. coli* deletion strains using the single gene knock-out strains as donors of a F'-plasmid carrying a tetracycline resistance gene and measured its transfer rate into recipient cells. We identify as many as 90 chromosomal genes required for fast transmission of antibiotics resistance by conjugation.

Conclusions

These chromosomal factors are prime targets for novel drug development initiatives aiming to delay conjugation and the spread of antibiotic resistance genes in combination therapy. We are currently repeating the screen with clinical and environmental resistance plasmids of different incompatibility groups to find common potential targets for therapeutic exploitation.

FEMS7-0419
Physiology / Biochemistry / Molecular Microbiology - Part II

MODULATION AND ANALYSIS OF THE ACTIVITY OF RECOMBINANT STAPHYLOCOCCUS SAPROPHYTICUS GTC1 LIPOLYTIC ENZYMES

A. Gricajeva[1], *L. Kalėdienė*[1]
[1]*Vilnius University, Life Sciences Center- Institute of Biosciences, Vilnius, Lithuania*

Backgrounds

Lipases of *Staphylococcus saprophyticus* are implicated as possible virulence determinants in pathogenesis during infections and, nevertheless, they can also be considered as potential industrial biocatalysts. Only fragmented information is known about *S. saprophyticus* as a biotechnological tool. In the present work we found that *S. saprophyticus* GTC1 exhibits high lipolytic activity and depending on environmental *in vitro* conditions secretes up to six lipolytic enzymes (Lip).

Objectives

- *In silico* analysis of *S. saprophyticus* GTC1 *Lip* genes and achievement of their recombinant forms;
- Increase of activity of the Lip enzymes;
- Determination of biochemical properties of achieved derivatives comparing with free forms.

Methods

Lip genes were cloned and expressed in *E. coli* DH5α / BL21 (DE3), respectively, using pET26(+)b. Positive targets were purified by immobilized metal affinity chromatography. Modulation of the recombinant Lip enzymes activity was achieved by non-conventional hydrophobic adsorption on octyl-Sepharose beads, chemical modulation and bioimprinting with surfactants techniques. Biochemical properties of the derivatives as well as V_{max}, K_M, k_{cat} and k_{cat}/K_M were determined using spectrophotometric assay with *p*-NP substrates. Regioselectivity (with 1,3-dipalmitoyl-2-oleoylglycerol substrate), hydrolysis and transesterification of natural fats were determined using thin layer chromatography.

Conclusions

Immobilization and bioimprinting of lipolytic enzymes resulted in the hyperactivation of the target proteins due to maintenance of their open lid forms. Activity of such enzymes became no longer dependent on the medium conditions. Modulation experiments resulted not only in hyperactivation but also change of enzymes selectivity. Such enzymes can be proposed as highly valuable catalysts with rationally designed properties. Their utilization can greatly boost many biotechnology-based industries.

FEMS7-0499
Physiology / Biochemistry / Molecular Microbiology - Part II

BIOFILM MATRIX PROTEINS ARE ESSENTIAL FOR GROWTH ON ALKANES AND LIPIDS

R. Grimaud[1], H. Ennouri[1,2], F. Hakil[1], P. Sivadon[1], P. Branchu[3], M. Naïtali[3], R. Oueslati[2], P. d'Abzac[1], J. Desbrières[1]
[1]IPREM UMR CNRS 5254 Université de Pau et des Pays de l'Adour, Microbiology and Environment, Pau Cedex, France
[2]Université de Carthage- Faculté des sciences de Bizerte,
Unité d'Immunologie- Microbiologie Environnementale et Cancérogenèse, Bizerte, Tunisia
[3]MICALIS- UMR 1319- INRA AgroParisTech,
Equipe Bioadhésion- Biofilm et Hygiène des Matériaux B2HM, Massy, France

Backgrounds

Due to their very low water solubility, hydrocarbons and lipids are weakly bioavailable. Despite this obstacle, many microbes are able to assimilate them by means of specialized strategies such as biofilm formation at the hydrocarbon/lipid-water interface so called oleolytic biofilms.

Objectives

In order to determine whether the extracellular polymeric substances (EPS) constituting the oleolytic biofilm matrix are involved in hydrocarbons/lipids assimilation, we undertook a characterization of the EPS of a biofilm of *Marinobacter hydrocarbonoclasticus* developing at the alkane water interface.

Methods

Matrix proteins were identified by proteomic analysis and their participation in oleolytic biofilm determined by proteolysis. The role of secreted proteins was assessed with a type-2 secretion system (T2SS) mutant.

Conclusions

The extracted matrix was largely composed of proteins typically cytoplasmic such as translation factors and chaperones, and a lesser amount of proteins of unknown function that are predicted extra-cytoplasmic. Matrix proteins were found mandatory for the development of biofilms on alkanes, lipids and polystyrene. Exo-proteins secreted through the T2SS were shown to be essential for the formation of oleolytic biofilms on both alkanes and triglycerides. The T2SS effector involved in biofilm formation on *n*-hexadecane is likely involved in the mass transfer, capture or transport of alkanes. We propose that *M. hydrocarbonoclasticus* uses cytoplasmic proteins possibly released by a regulated auto-lysis mechanism to form a proteinaceous matrix and dedicated proteins secreted through the T2SS to act specifically in the assimilation pathways of hydrophobic substrates.

FEMS7-1327
Physiology / Biochemistry / Molecular Microbiology - Part II

PHYSIOLOGICAL PORTRAIT OF PECTOBACTERIUM ATROSEPTICUM UNDER IN PLANTA CONDITIONS IN TERMS OF TRANSCRIPTOME PROFILING

R. Gubaev[1], V. Gorshkov[1,2], C. Nuriakhmetova[3], S. Tatarkin[1], N. Gogoleva[1], Y. Gogolev[1,2]
[1]Kazan Institute of Biochemistry and Biophysics - RAS, Molecular Biology, Kazan, Russia
[2]Kazan Federal University, Biochemistry and Biotechnology, Kazan, Russia
[3]Kazan Federal University, Genetics, Kazan, Russia

Backgrounds

Necrotrophic pectobacteria cause severe parenchymatous rots in plants. Expression of symptoms is preceded by latent infection when pathogen predominantly inhabits xylem vessels, prepares host for further colonization, and behaves as biotroph. Herewith, the pathogen reprograms its physiology from “outside” to “inside the host” status. However, general picture of such “switch” remains to be developed.

Objectives

We aimed to get better insight into physiological portrait of pectobacteria during the initial and advanced stages of plant colonization.

Methods

We took advantage of RNA-Seq approach, high efficient for a global view on cell physiology, but to out knowledge not previously applied for any of plant pathogenic bacteria under *in planta* conditions. Transcriptomes of pectobacteria *in planta* at initial and advanced stages of plant colonization were compared with those of different model cultures *in vitro*. In order to create demonstrative picture of cell physiology from the lists of the revealed differentially expressed genes (DEGs), original multiple functional classification of DEGs was performed using KEGG and BioCyc sources as well as various specialized databases (e.g. CAZy, REBASE, TAD, etc.).

Conclusions

Under *in planta* vs. *in vitro* conditions several alterations in general metabolism (e.g. switching from aerobic metabolism to fermentation, amino acid and carbon metabolism, etc.) were revealed. Many pathways related to virulence and adaptation were up-regulated *in planta*. Differential features of pectobacteria during different stages of infection (e.g. phytotoxin coronatine metabolism and type III secretion system, etc.) were detected. Further details on pectobacteria physiology *in planta* will be discussed during presentation. This study was supported by RSF (15-14-10022).

FEMS7-2932
Physiology / Biochemistry / Molecular Microbiology - Part II

A NEW COLISTIN RESISTANCE GENE HOMOLOGOUS FOUND IN WASTEWATER METAGENOME

A. Gundogdu[1,2], *M.G. Onal*[2,3], *A. Ulu-Kilic*[4], *U. Nalbantoglu*[2,5]
[1]*Erciyes University, Microbiology and Clinical Microbiology, Kayseri, Turkey*
[2]*Erciyes University, Genome and Stem Cell Center GENKOK, Kayseri, Turkey*
[3]*Erciyes University, Medical Genetics, Kayseri, Turkey*
[4]*Erciyes University, Clinical Microbiology and Infectious Diseases, Kayseri, Turkey*
[5]*Erciyes University, Computer Engineering, Kayseri, Turkey*

Backgrounds

Colistin, a drug of last resort, has been used in order to cope with multi-drug resistant pathogens. A recently defined plasmid mediated colistin resistance gene (*mcr-1*) raised attention, since the mobilty of this gene means rapid dissemination of colistin resistance. Despite the increasing colistin resistance, *mcr-1* or recently discovered variants have not been detected in Turkey. This brings the hypothesis that another mcr-variant might be present in the geography.

Objectives

This study aimed to search for a mcr-like gene in the wastewater microbiome, which is considered to be the hot-spots of antibiotic resistance genes, using next-generation sequencing technologies.

Methods

Three weekly samples from wastewaters (STP and hospital) were collected. Total DNA isolation was performed using commercial kits, and the sequencing was done using Illumina-NextSeq500 technology. Sequencing reads similar to *mcr-1* and *mcr-2* genes were searched within the metagenome using Blastx. The detected reads were subject to fragment assembly using Cap3-assembler to obtain longer fragments. Primer sets were designed on the assembled contigs to employ genome walk for further sequencing of the uncovered gene regions. Amplified regions were sequenced using Sanger sequencing and scaffolded with the previous assembly.

Conclusions

A new mcr-homologous gene was sequenced from wastewater samples. Since no mcr genes responsible for colistin resistance in Turkey was detected before, this newly discovered gene is an interesting candidate for the dissemination agent of the colistin resistance in the region. Although this gene resembles its recently defined homologs, functional validation should be conducted to reveal if this variant is actually responsible for colistin resistance.

FEMS7-1462
Physiology / Biochemistry / Molecular Microbiology - Part II

TAIL-ANCHORED INNER MEMBRANE PROTEIN ELAB INCREASES STRESS RESISTANCE WHILE REDUCING PERSISTENCE IN ESCHERICHIA COLI

Y. Guo[1], X. Liu[1], B. Li[1], J. Yao[1], T.K. Wood[2], X. Wang[1]
[1]Chinese Academy of Sciences- South China Sea Institute of Oceanology, LMB, Guangzhou-, China
[2]Pennsylvania State University, Department of Chemical Engineering, University Park, USA

Backgrounds

Host-associated bacteria, such as *Escherichia coli*, often encounter various host-related stresses such as nutritional deprivation, oxidative stress and temperature shifts. There is growing interest in searching for small endogenous proteins that mediate stress responses.Here, we characterized a small C-tail anchored inner membrane protein ElaB in *E. coli*. ElaB belongs to a class of tail-anchored inner membrane proteins with a C-terminal transmembrane domain but lacking an N-terminal signal sequence for membrane targeting. Proteins from this family have been shown to play vital roles such as membrane traffic and apoptosis in eukaryotes.

Objectives

To uncover the roles of this family protein in prokaryotes.

Methods

We found that transcription of *elaB* is induced in the stationary phase, and sigma factor RpoS regulates *elaB* transcription by binding to the promoter of *elaB*. Moreover, ElaB protects cells against oxidative stress and heat shock stress. However, unlike membrane peptide toxins TisB and GhoT, ElaB does not lead to cell death, and deletion of *elaB* greatly increases persister cell formation. We also demonstrate that ElaB protects the integrity of membrane and inhibits the flux of H_2O_2 into cells. The *elaB* mutant strain had wrinkled thinner membrane and more susceptible to oxidative stress. Using granulocytopenic mouse model, survival was decreased in the Δ*elaB* strain, suggesting that ElaB might participate in pathogen-host interaction.

Conclusions

We demonstrate that targeting C-tail anchored inner membrane proteins for treating bacterial infections might be problematic, since, although disruption of membrane proteins can reduce stress resistance, it can also lead to deleterious effects such as increased persistence.

FEMS7-0661
Physiology / Biochemistry / Molecular Microbiology - Part II

FUNCTIONAL CHARACTERIZATION OF BAS2108-2109 TWO COMPONENT SYSTEM OF B. ANTHRACIS INVOLVED IN REGULATION OF PROTEASES

V. Gupta[1], P. Gulati[2], R.B. Bhatnagar[1]
[1]jawaharlal nehru university, Biotechnology, New Delhi, India
[2]Maharshi Dayanand University, Microbiology, Rohtak- Haryana, India

Backgrounds

Bacillus anthracis is the causative agent of anthrax, a bioweapon. This bacteria have a complex secretome which includes secreted proteases along with other virulence factors. Although protease sevretion varies under different conditions, there are no reports about regulatory mechanisms governing protease expression. Pathogens commonly use two component system (TCS) for environment sensing and regulation of virulence factors. The genome of B. anthracis encodes 41 such TCS pairs, however, the role of any TCS pair in regulation of its proteases is not known.

Objectives

In silico screening for a putative TCS regulating protease expression in *B. anthracis.* Biochemical characterization of TCS and to study its role in virulence.

Methods

TCS screening was performed using homology search. The identified TCS was biochemically characterized. Their expression was analyzed using qRT-PCR. DNA-protein interaction was analyzed by electrophoretic mobility shift assay. Multiple sequence alignment was done to predict conserved residues and validated by mutational studies.

Conclusions

The present study have identified BAS2108-2109 as a putative homolog of protease regulator, DegUS of *B. subtilis*. It is a functionally active TCS, where BAS2108, a histidine kinase, autophosphorylates and transfers phosphate group to BAS2109, the response regulator. The expression of *BAS2109* was increased under simulation of host milieu. BAS2109 was established as the transcriptional regulator for different genes of *B. anthracis*, particularly proteases. Lys167, Thr179 and Thr182 residues were significant for the DNA binding activity of BAS2109. Therefore, this report establishes BAS2108-2109 as a canonical TCS pair which is involved in the regulation of expression of proteases in *B. anthracis.*

FEMS7-0973
Physiology / Biochemistry / Molecular Microbiology - Part II

ROLE OF CSRA GLOBAL REGULATOR IN UROPATHOGENIC E. COLI BIOFILMS STRUCTURE

T. Hallaert[1], E. Seyll[1], S. Zedek[1], D. Monteyne[1], P.M. David[1], L. Van Melderen[1]
[1]Université Libre de Bruxelles - IRMW, Faculté des Sciences, Brussels, Belgium

Backgrounds

The CsrA protein is a global post-transcriptional regulator controlling carbon fluxes and social behaviors in bacteria. Independent studies have shown that CsrA regulates different processes involved in biofilm formation such as flagella synthesis, exopolysaccharides production or c-di-GMP homeostasis.

Objectives

Internal organization of K-12 *Escherichia coli* biofilms had recently been described at cellular resolution using macrocolony model and scanning electron microscopy (SEM) by the group of R. Hengge. Our objective is to decipher the role of CsrA into biofilm organization by characterizing biofilms formed by a mutant deleted for *csrA* in *E. coli* CFT073.

Methods

Using genetics and biochemical approaches and scanning electron microscopy, we analyzed at macro- and microscopic level a collection of mutants deleted for *csrA* and other genes encoding proteins involved in production of extracellular structures such as curli, N-acetylglucosamine (PGA), flagella or participating to c-di-GMP homeostasis.

Conclusions

SEM observations of wild-type macrocolonies revealed very organized structures such as vertical pillars and differentiated horizontal layers. In a mutant deleted for *csrA*, macrocolony organization is lost. We also showed that deletion of *csrA* abolishes negative regulation of the YcdT c-di-GMP cyclase, leading to an increase of intracellular c-di-GMP concentration. In turn, both cellulose and PGA are highly overproduced. We propose that this abundant extracellular matrix is produced anarchically thereby hindering macrocolony internal organization.

FEMS7-2404
Physiology / Biochemistry / Molecular Microbiology - Part II

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED IN THE COMMUNITY, CARRIERS OF THE MECC GENE.

A. Hamdan-Partida[1], B. Sánchez-Mier[1], S. González- García[1], J. Bustos-Martínez[1]
[1]Universidad Autónoma Metropolitana Xochimilco, Atención a la Salud, Mexico City, Mexico

Backgrounds

Staphylococcus aureus is a Gram-positive bacterium that has the ability to express a wide variety of virulence factors, through which it produces various diseases. In particular, methicillin-resistant *S. aureus* (MRSA) strains, which present the *mecA* gene responsible for resistance to methicillin, since it encode a penicillin binding protein 2a (PBP2a), that has low affinity by β-lactam antibiotics. Recently, a new gene for resistance to methicillin, *mecC*, was discovered, which has not been reported in strains isolated in Mexico.

Objectives

The objective of this work was to know if MRSA strains isolated in the population of Mexico City, carry the *mecC* gene .

Methods

In the present study, pharyngeal and nasal exudates of 1800 people, aged 1 to 80 years old, were performed in day care centers, schools, nursing homes and factories in Mexico City. Isolation and identification of staphylococci were performed by established microbiological methods. Sensitivity to methicillin was performed using the MIC-oxacillin test. Identification of *mecA* and *mecC* genes were performed by the PCR technique.

Conclusions

1135 *S. aureus* strains were isolated, and only 135 strains were MRSA. Most of the MRSA strains were carries of the *mecA* gene and only 7 srains MRSA were carriers of the *mecC* gene.

This is of great importance since it is the first report in Mexico of MRSA strains carrying the gene *mecC*.

FEMS7-2421
Physiology / Biochemistry / Molecular Microbiology - Part II

ANTIMICROBIAL EFFECT OF SILVER NANOPARTICLES AGAINST VIBRIO FLUVIALS

A. Hamdan-Partida[1], J. Meneses-Márquez[1], J. Bustos-Martínez[1], C. Monroy-Dosta[2]
[1]Universidad Autónoma Metropolitana Xochimilco, Atención a la Salud, Mexico City, Mexico
[2]Universidad Autónoma Metropolitana Xochimilco, El Hombre y su Ambiente, Mexico City, Mexico

Backgrounds

Nanotechnology is a field of research in nanoparticles due to their properties, some of these nanoparticles have an antimicrobial effect, in particular silver nanoparticles. The use of silver as an antimicrobial has been since ancient times. In aquaculture, ornamental fishes have expressed bacterial diseases, some of these bacteria have resistance to different types of antibiotics. The aquaculture industry are looking for new alternatives for the control of these bacteria.

Objectives

The aim of this work was determine the antimicrobial effect of silver nanoparticles (AgNPs) against *Vibrio fluvialis*. Characterize the nanoparticles, and determine the minimum inhibitory concentration (MIC).

Methods

The AgNPs were synthesized at different concentrations (10, 100, 250, and 500 ppm) using sodium citrate as a reducing agent. To determine the minimum inhibitory concentration (MIC), the bacteria *V. fluvialis* were grown in Muller Hilton broth for 24 h to obtain a concentration of 0.5 McFarland, and were mixed with serial dilutions of different concentrations of AgNPs.

Conclusions

The result showed that the MIC of the AgNPs to *V. fluvialis* was between 7.81 and 15.62 ppm. We concluded that our AgNPs showed antimicrobial activity against *V. fluvialis*. We recommended continue studying the effect of AgNPs in other pathogenic microorganisms which affect the health of the ornamental fishes.

FEMS7-3275
Physiology / Biochemistry / Molecular Microbiology - Part II

DETECTION OF B -LACTAMASE ENZYMES USING CONVENTIONAL AND MOLECULAR METHODS

H. HAMED[1], E. el sawy[1], A. gaber[1]
[1]urology and nephrology center, microbiology, mansoura, Egypt

Backgrounds

Resistance to antimicrobials is a serious clinical problem, with more than 70% of the bacteria that cause hospital-acquired infections resistant to at least one of the drugs that are currently used for treatment of infections, β-Lactam antibiotics remain the most commonly used antibacterial agents.

Objectives

Detection methods for β-lactamases are nitrocefin test, Phenol red method, Iodometric method, and Double-Disc Test and PCR.

Methods

97 beta-lactam resistant bacterial strains 50 *E.coli* and 47 as *Klebsiella pneumoniae* were studied. The Combined disc method, Etest ESBL strips, Phenol red method, Iodometric method, and nitrocefin tests were performed. DNA extraction of the resistant strains was performed, followed by polymerase chain reaction test(PCR) for detection of TEM and SHV β-lactamase genes.

80 strains gave positive results for Etest ESBL strips, combined disc method, Phenol red test, Iodometric test, and nitrocefin tests, while 17 strains gave negative results. 8 strains(4 *E.coli* & 4 *Klebsiella pneumoniae*) were positive for TEM gene and SHV gene; 27 strains(14 *E.coli* & 13 *Klebsiella pneumoniae*) were positive for TEM gene only; 28(15 *E.coli* & 13 *Klebsiella pneumoniae*) strains were positive for SHV gene only; while 34 strains(17 *E.coli* & 17 *Klebsiella pneumoniae*) were negative for the two genes.

Conclusions

The development and spread of extended-spectrum β-lactamases(ESBLs) have most likely been caused by the overuse of expanded spectrum cephalosporins in the hospital setting. Numerous methods have been proposed for the detection of ESBLs in clinical isolates. Regardless of the method used for detection, none of the methods that rely on phenotypic expression of the β-lactamase will detect every ESBL-producing isolate. Nevertheless, increased awareness of the ESBL problem among clinical microbiology laboratory and infection control personnel will help in the interpretation of these tests. Strains expressing ESBLs will present a host of challenges for clinical microbiologists and clinicians alike as we head into the 21st century.

FEMS7-1778
Physiology / Biochemistry / Molecular Microbiology - Part II

DETERMINATION OF TRANSFER ORIGIN OF INCA/C PLASMIDS

A. Hegyi[1], M. Szabó[1], F. Olasz[1], J. Kiss[1]
[1]NARIC Agricultural Biotechnology Institute, Genetics, Gödöllő, Hungary

Backgrounds

The plasmids of incompatibility group A/C are often responsible for multi-drug resistance (MDR) of enteric bacteria. IncA/C plasmids spread efficiently due to their broad host-range and effective conjugative transfer system, thus they are major factors in dissemination of MDR phenotype in pathogenic bacteria. Although more than 140 family members have been identified, the biology of their spread received more attention only recently.

Objectives

Our goal was to identify and analyse the origin of transfer of three IncA/C plasmids – R55, R16a and IP40a.

Methods

R55 plasmid library was created. The minimal length of the presumed oriTs required for fully active transfer of a non-conjugative cloning vector have been determined and analysed by directed deletions and conjugation tests.

Conclusions

We detected two mobilizable regions – named as $oriT_1$ and $oriT_2$ – whose locations on the plasmid are quite different. We found that deletion of $oriT_2$ completely abolished the conjugation of the IncA/C plasmids confirming that it is a functional transfer origin. The $oriT_2$ locates between divergent genes, and one of them, ORF001, is also required for the transfer of IncA/C plasmids. Unlike $oriT_2$, deletion of $oriT_1$ had no significant effect on the conjugation. Furthermore, high transfer frequency observed with a non-mobile plasmid carrying the cloned $oriT_1$ region was due to the frequent cointegration of IncA/C and $oriT_1$-plasmid via a short sequence in $oriT_1$. Thus, the sequence named as $oriT_1$ rather seems to be a recombination hotspot than a real transfer origin.

FEMS7-1450
Physiology / Biochemistry / Molecular Microbiology - Part II

STUDY OF A VIBRIO CHOLERAE DETERMINANT OF PROPER MORPHOLOGY

S.B. Hernández[1], M.K. Waldor[2], F. Cava[1]
[1]MIMS- Umeå University, Molecular Microbiology, Umeå, Sweden
[2]Harvard Medical School, Department of Microbiology and Immunobiology, Boston- Massachusetts, USA

Backgrounds

The bacterial cell wall is a net-like structure composed mainly of peptidoglycan (PG). PG is essential for maintenance of cell shape and survival, therefore inhibition of PG synthesis and certain cell wall damages usually lead to cell death.
During a morphological screening, we found a locus of *Vibrio cholerae* whose mutation results in the formation of round, osmotically sensitive cells in stationary phase. Stationary phase-specific loss of proper morphology has been previously described in *V. cholerae* cells impaired in the PBP1A (Penicillin Binding Protein 1A) and associated regulatory proteins.

Objectives

Characterize the enzymatic activity and the biological function of the new protein found to be involved in PG homeostasis of *V. cholerae*.

Methods

A transposon insertion library of *V. cholerae* was used for the morphological screening using microscopy.
Analysis of the PG structure of the mutant and characterization of the enzymatic activity of the protein were performed.
Suppressor mutants were studied by: i) whole genome sequencing of isolated spontaneous suppressors; ii) transposon insertion sequencing of suppressors isolated under low osmolarity conditions.
To study the biological role of the protein of interest, a Tn-seq experiment was performed for the identification of synthetically lethal mutations.

Conclusions

A number of evidences suggest that the role of the protein under study in morphogenesis and PG homeostasis occurs in a PBP1A independent-fashion: i) is cytosolic protein; ii) development of spheres in the mutant is independent of NCDAA production; iii) its mutation is synthetically lethal with PBP1A; iv) the mutant exhibits a high generation rate of suppressor mutants.

FEMS7-0825
Physiology / Biochemistry / Molecular Microbiology - Part II

IDENTIFICATION OF NOVEL INTERACTION PARTNERS OF THE PERSISTER REGULATOR OBG

P. Herpels[1], J. Rayo Company[2], A. Economou[2], N. Verstraeten[1], J. Michiels[1]
[1]KU Leuven, Department of Microbial and Molecular Systems M²S, Leuven, Belgium
[2]KU Leuven, Department of Microbiology and Immunology, Leuven, Belgium

Backgrounds

Chronic infections that are recalcitrant to treatment with antibiotics are posing a major threat to human health. This recalcitrance is partly caused by so-called persister cells. Persisters comprise a small (typically < 0.1%) fraction of transiently antibiotic-tolerant cells that are able to survive prolonged exposure to antibiotic treatment. Although the clinical importance has been demonstrated, the precise molecular mechanisms underlying persistence are not fully unravelled.

Objectives

Previous research in our group has demonstrated a central role for the conserved GTPase Obg in mediating persistence. In the current project, direct interaction partners of Obg are identified to further unravel the Obg-persistence pathway and select specific targets that influence persister formation.

Methods

An innovative photo-crosslinking technique is used to identify novel direct interaction partners of *Escherichia coli* Obg. The unnatural photo-reactive amino acid *p*-benzoyl-L-phenylalanine (*p*Bpa) is incorporated at specific locations on the surface of the Obg protein. Following UV radiation, the carbonyl oxygen of *p*Bpa crosslinks to carbon-hydrogen bonds of molecules within a radius of 3 angstrom. Interaction partners of Obg are identified using high resolution liquid chromatography-mass spectrometry. Subsequently, specific proteins and corresponding genes are tested for their involvement in persistence.

Conclusions

Identification of Obg-interaction partners contributes to the full understanding of the pathway. It is expected that targeting Obg-mediated persistence will significantly reduce the number of persister cells in bacterial populations, thereby facilitating clearing of infections by conventional antibiotics.

FEMS7-0296
Physiology / Biochemistry / Molecular Microbiology - Part II

CAN THESE GENES STRETCH ANYMORE?! – ON EXPERIMENTAL EVOLUTION OF CHLAMYDIAE

P. Herrera[1], L.N. Schuster[1], M. Zojer[2], H. Na[1], J. Schwarz[1], F. Wascher[1], T. Rattei[2], M. Horn[1]
[1]University of Vienna, Division of Microbial Ecology, Vienna, Austria
[2]University of Vienna, Division of Computational Systems Biology, Vienna, Austria

Backgrounds

Experimental evolution approaches have been essential for understanding evolutionary mechanisms. However, our knowledge of evolutionary dynamics of strictly host-dependent bacteria is largely based on comparative genomics of closely-related species. The phylum Chlamydiae is comprised exclusively of bacteria with an obligate intracellular lifestyle. Their associations with eukaryotic hosts are remarkably diverse: They are major pathogens of many animals and occur ubiquitously as protist symbionts.

Objectives

Our laboratory has been conducting an ongoing long-term evolution experiment (LTEE) using the chlamydial symbiont *Protochlamydia amoebophila* and its amoeba host. The main aim is to improve our understanding of the evolutionary dynamics and genomic/molecular basis of the intracellular lifestyle of chlamydiae with respect to host adaptation, interaction, and character of the symbioses.

Methods

We have established replicate *Protochlamydia* populations thriving in their *Acanthamoeba* hosts under two temperature regimes for 500 generations (38 months). Pool sequencing was carried out at selected time points, and variant detection was performed using a novel work flow to detect low abundant variants in high-coverage sequence pools. Infectivity assays were carried out to compare ancestral and selected evolved populations.

Conclusions

The observed substitution rate was surprisingly similar to those of free-living microbes, with a significant increase at 30°C compared to 20°C incubation temperature. Non-synonymous substitutions were more frequent at 30°C, indicating a higher level of positive selection. Infectivity assays revealed that 30°C populations exhibited reduced virulence. In summary, we were able to track evolutionary dynamics of a chlamydia symbiont within its protected intracellular niche, providing a better understanding of their adaptation to elevated temperature.

FEMS7-1994
Physiology / Biochemistry / Molecular Microbiology - Part II

DEVELOPMENT OF FLUORESCENCE BASED BIO-REPORTER SYSTEM FOR DETECTING ANTIBIOTIC-INDUCED MISTRANSLATION

M. Hinnu[1], *M. Putrinš*[1], *K. Kogermann*[2], *T. Tenson*[1]
[1]*University of Tartu, Institute of Technology, Tartu, Estonia*
[2]*University of Tartu, Institute of Pharmacy, Tartu, Estonia*

Backgrounds

Errors in protein translation are common and can be beneficial for bacteria. It has been shown that increased mistranslation can modify the susceptibility to antibiotics. On the other hand, antibiotics themselves can increase mistranslation.

Objectives

The aim of our study was to develop a fluorescence based bio-reporter strain, which could be used to estimate the prevalence of mistranslation during infection and antibiotic treatment. Possible future applications include guiding the development of better dosing regimens for existing antibiotics and the design and development of new antibiotics.

Methods

Fluorescence based mistranslation reporter system was developed and studied in uropathogenic *Escherichia coli* strain CFT073. The reporting system uses green fluorescent protein (GFP) as a mistranslation indicator, and red fluorescent protein mCherry as a control protein for expression. Therefore, the ratio of green and red fluorescence can be used as an indicator of mistranslation. Frameshift and nonsense mutants of GFP were constructed. Fluorescence of bulk bacterial cultures was measured by plate-reader and fluorescence of single cells by flow cytometer. Effects of different antibiotics were studied.

Conclusions

Our reporter enabled detecting frameshifting in stationary phase cells. With the addition of aminoglycosides, single cell analysis showed an increase in non-fluorescent bacterial population, which might indicate death of bacteria in sub-MIC antibiotic concentrations. The system still needs to be developed further for more extensive studies with antibiotics.

FEMS7-2758
Physiology / Biochemistry / Molecular Microbiology - Part II

BIOCHEMICAL SURVEY OF THE RID PROTEIN FAMILY DEMONSTRATES DEAMINASE ACTIVITY IS CONSERVED AMONG SUBFAMILIES

K. Hodge-Hanson[1], *D. Downs*[1]
[1]*University of Georgia, Microbiology, Athens, USA*

Backgrounds

The Rid (YjgF/YER057c/UK114) protein family is conserved across the three domains of life and consists of eight subfamilies: RidA and Rid1-7. The archetypal member, RidA from *Salmonella enterica* is a **R**eactive **I**ntermediate **D**eaminase that hydrolyzes enamine/imine intermediates generated as catalytic intermediates in bacterial metabolism. The importance of RidA is reflected in the various processes affected in its absence in a number of organisms including bacteria, plants and yeast. Production of the reactive intermediates, as well as the consequences of lacking RidA are both linked to the essential cofactor pyridoxal 5'-phosphate (PLP). Our understanding of the remaining subfamilies is in its infancy. Some genomes encode multiple Rid proteins such as the opportunistic pathogen *Pseudomonas aeruginosa* with nine encoded Rid proteins, representing multiple subfamilies.

Objectives

This work was initiated to probe functional overlap between Rid proteins from subfamilies Rid1-3 in an effort to understand the role(s) of enamine/imine intermediates in bacterial metabolism.

Methods

A biochemical-genetic approach was taken; Rid1-3 subfamily proteins from various gamma-Proteobacteria were compared to RidA *in vivo* using the best-studied RidA paradigm in *S. enterica.* Purified Rid proteins were assessed for the ability to deaminate a wide range of short-lived intermediates generated *in situ* from both PLP-and FAD-dependent enzymes.

Conclusions

A biochemical-genetic approach was taken; Rid1-3 subfamily proteins from various gamma-Proteobacteria were compared to RidA *in vivo* using the best-studied RidA paradigm in *S. enterica.* Purified Rid proteins were assessed for the ability to deaminate a wide range of short-lived intermediates generated *in situ* from both PLP-and FAD-dependent enzymes.

FEMS7-0288
Physiology / Biochemistry / Molecular Microbiology - Part II

CROSS-RECOGNITION OF PROMOTERS BY ALTERNATIVE STRESS-RESPONSE SIGMA FACTORS IN STREPTOMYCES COELICOLOR A3(2)

D. Homerova[1], B. Sevcikova[1], B. Rezuchova[1], R. Novakova[1], J. Kormanec[1]
[1]Institute of Molecular Biology Slovak Academy of Sciences, gene expression, Bratislava, Slovak Republic

Backgrounds

Bacteria are in their natural habitats exposed to various stresses. The stress response is regulated mainly by stress-response sigma factors of RNA polymerase. In *Bacillus subtilis*, the activity of such sigma factor SigB is regulated by the anti-sigma factor RsbW, anti-anti-sigma factor RsbV, and by two PP2C phosphatases, RsbP and RsbU. However, the genome of Gram positive soil bacterium *Streptomyces coelicolor* contains genes for 65 sigma factors including 9 close homologues of SigB (SigB, F, G, H, I, K, L, M, N), 45 homologues of RsbW, 17 homologues of RsbV, and 44 homologues of RsbU/RsbP that indicates rather complex picture of regulation comparing to *B. subtilis*. We previously characterised several of these SigB homologues in *S. coelicolor* and found their dual role in morphological differentiation and osmotic stress response [1].

Objectives

Characterization of the recognition of promoters by nine SigB homologues using two plasmid system. Verification of their dependence in sigma factor gene mutants using *lux* reporter system.

Methods

E. coli two plasmid system is described in [2]. *In vivo* activity of promoters was investigated using a *lux* reporter system [3].

Conclusions

1, *S. coelicolor* SigB homologues cross-recognize several promoters in *E. coli* two-plasmid system.

2, *In vivo* activity of the promoters was differentially dependent upon these sigma factors during differentiation and after osmotic stress conditions.

Acknowledgements. This work was supported by the VEGA grant 2/0002/16 from Slovak Academy of Sciences.

References.

1. Kormanec J, Sevcikova B, Novakova R, Homerova D, Rezuchova B, Mingyar E (2016) Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria, John Wiley & Sons, 321-336.

2. Novakova R, Sevcikova B, Kormanec J (1998) *Gene* 209, 43-50.

3. Craney A, Hohenauer T, Xu Y, Navani NK, Li Y, Nodwell J (2007) *Nucleic Acids Res.* 35, e46.

FEMS7-1480
Physiology / Biochemistry / Molecular Microbiology - Part II

A COMPETITOR OR SCAVENGER: WHAT ROLE DOES GSKIP PLAYS IN THE WNT SIGNALING PATHWAY?

Y.R. Hong[1]
[1]*Kaohsiung medical university, Biochemistry, Kaohsiung, Taiwan R.O.C.*

Backgrounds

A-Kinase Anchoring proteins (AKAPs) are a family of PKA scaffolding proteins. It allows PKA to phosphorylate other signaling molecules and achieve spatiotemporal modulation of their activities through protein-protein interaction. GSKIP is an AKAP that promotes PKA phosphorylation of GSK3β.

Objectives

First, we found that wild-type GSKIP attenuates yeast growth but the process can be revoked by either PKA or GSK3β binding site mutants. Bioinformatics study, on the other hand, revealed that GSKIP contains the evolutionary conserved DUF727, which possesses both PKA and GSK3β binding sites in vertebrate, but only site for GSK3β in invertebrate. This could explain why human GSKIP significantly decrease growth rate might due to yeast lacking PKA binding site and affected. We also found that both PKA and GSK3βsites affect the binding, in various forms of GSK3β in yeast two-hybrid assays. However, pre-incubation of yeast cells with either PKA inhibitor H89 or GSK3βinhibitor LiCl had no effect on growth or binding. We also showed that the effect of GSKIP on β-catenin is highlighted by its functioning as a competitor or scavenger in a Wnt3a stimulus; it recruits the GSK3β away from the destruction complex and simultaneously brings PKA to form a new PKA/GSKIP/GSK3β/β-catenin complex.

Methods

GSKIP was studied through yeast growth upon GSKIP transfection, yeast two-hybrid, cell line-based assay, knock-out mice, 2D-DIGE combined mass spectrometry and bioinformatics.

Conclusions

Our findings highlight an essential complex of a cAMP/PKA/β-catenin axis by GSKIP- and GSK3β-mediated anchoring in modulating β-catenin phosphorylation via Wnt signaling.

FEMS7-0374
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERIZATION OF THE SEVEN NAD+-DEPENDENT ALCOHOL DEHYDROGENASES IN ACINETOBACTER BAUMANNII

M.C. Hsieh[1], *G.H. Lin*[1]
[1]*Tzu Chi University, Department of Microbiology, Hualien, Taiwan R.O.C.*

Backgrounds

Acinetobacter baumannii is an aerobic Gram-negative bacteria, and this opportunistic human pathogen causes a seriously nosocomial infections. To prevent nosocomial infections, ethanol is commonly used for surface cleaning and disinfecting surgical devices. In previous study showed that low doses of ethanol not only as a carbon source but also as a bacterial signaling molecule which can enhance the bacterial growth and increase salt tolerance of *A. buamanii*.

Objectives

In alcohol metabolism, alcohol dehydrogenases (ADH) catalyze the reversible oxidation of alcohols to aldehydes or ketones. In order to understand the seven ADHs are functionally different and find out the different kinetic characteristics in *Acinetobacter baumannii*.

Methods

In silico analysis, *A. baumannii* contain seven ADHs which belong to NAD^+-dependent ADHs. We constructed *Abadh*s mutants by marker-less mutation and cultured *Abadh*s mutants in different concentration alcohols as sole carbon source. Although constructed *Abadh*s expression strain by *E.coli* and purified AbAdhs for enzyme activity.

Conclusions

AbAdh1, AbAdh2, and AbAdh7 are zinc-containing ADH. AbAdh3, AbAdh4, and AbAdh6 are iron-containing ADH. AbAdh5 is a short-chain ADH. *Abadh3* deletion mutants decrease 1-propanol metabolism. *Abadh4* deletion mutants cannot grow in ethanol, butanol, and 1-propanol as a single carbon source. These data seem like AbAdh4 is the most important ADH of alcohol metabolism in *A. baumannii*. Kinetics studies showed that iron-containing AbAdh3 are in need of iron for ethanol oxidation.

FEMS7-0556
Physiology / Biochemistry / Molecular Microbiology - Part II

FUNCTIONAL STUDY OF ORF58 (MAB_2101 HOMOLOGUE) IN MYCOBACTERIUM MASSILIENSE

S.T. Hu[1], P.H. Lin[1]
[1]National Yang-Ming University, Microbiology and Immunology, Taipei, Taiwan R.O.C.

Backgrounds

Two strains, R49R (rough) and R49S (smooth), of *M. massiliense* isolated from same patient were identified derived from same pedigree and displayed different phenotypes. Whole genome sequences analysis of these strains showed a 54-kb deletion within R49R genome. This 54-kb region is flanked with 19-mer direct repeats and comprises of 58 open reading frames (ORFs) including a putative recombinase ORF58 (Mab_2101 homologue). It is possible that ORF58 acts as a recombinase to cause 54-kb deletion at the 19-mer direct repeats, resulting in the phenotypic differences of these strains.

Objectives

This study aims to determine the function of ORF58.

Methods

The *orf58* driven by *ptr* promoter was overexpressed in R49S, which indeed resulted in some phenotypic differences, but no deletion of 54-kb. The ORF58 contains the putative resolvase, recombinase, and zinc ribbon domains. To investigate which domain is essential for the function of ORF58, the plasmid containing full-length or truncated gene was overexpressed in R49S. The results showed that only resolvase and zinc ribbon domains were essential. The resolvase of transposon *3* also plays a role in transcriptional regulation, suggesting that ORF58 is more likely a regulator rather than a recombinase. Indeed, the transcriptome analysis of R49S gene expression revealed that the overexpressed ORF58 induced some interesting genes. In addition, when the plasmid containing *orf58* was cured from R49S, the bacterial phenotype switched back, indicating that the ORF58 might not cause recombination event in bacterium.

Conclusions

It is concluded that ORF58 may function as a transcriptional regulator rather than a recombinase.

FEMS7-1884
Physiology / Biochemistry / Molecular Microbiology - Part II

DIET INDUCED MICROBIOTA AND M-CELL MODIFICATIONS IN RABBIT GUT ASSOCIATED LYMPHOID TISSUE (GALT)

R. Arrazuria[1], E. Molina[1], M. Serrano[1], M. Tello[1], I.A. Sevilla[1], E. Khafipour[2], V. Pérez[3], A. Hurtado[1], R.A. Juste[4], J.M. Garrido[1], N. Elguezabal[1]
[1]NEIKER-Tecnalia, Department of Animal Health, Derio, Spain
[2]University of Manitoba, Department of Animal Health, Winnipeg, Canada
[3]University of León, Department of Animal Health, León, Spain
[4]SERIDA, Department of Agriculture of the Regional Government of the Principality of Asturias, Deva, Spain

Backgrounds

The gut microbiome plays an important role in competitive exclusion of pathogens and in immunity development and maturation. Rabbit GALT is predominantly composed of two structures: the sacculus rotundus (SR) and the vermiform appendix (VA). M-cells are specialized epithelial cells covering the lymphoid follicles of the GALT that uptake antigens from the lumen and subsequently put them in contact with cells of the immune system.

Objectives

To evaluate modifications in the microbiota and in the M-cell population in GALT of rabbits fed regular or high fiber diet.

Methods

Microbial composition and the abundance of M-cells of VA and SR were studied in 10 New Zealand rabbits. Half of those rabbits were fed a regular diet during the whole experiment. In the remaining rabbits the regular diet was switched to a high fiber diet from weeks 7 to 11 and 23 to 27 of the beginning of the experiment. M-cells were identified by vimentin immunohistochemistry and analyzed using the IHC Profiler in ImageJ. The microbiota was studied by 16S rRNA sequencing. Data treatment was carried out following QIIME pipeline and analyzed in LefSe.

Conclusions

Immunohistochemistry analysis revealed that the number of M-cells was increased with dietary fiber in both sacculus rotundus and vermiform appendix ($p<0.05$). Analysis of microbial composition showed the overrepresentation of the family Dehalobacteriaceae in sacculus rotundus and the family Odoribacteriaceae and the genus *Butiricimonas* in vermiform appendix of animals fed with high fiber diet. These findings suggest that the hyperplasia of M-cells may be induced by dietary and microbial factors.

FEMS7-0490
Physiology / Biochemistry / Molecular Microbiology - Part II

EVOLUTION OF GLOBAL MODULATORS: A NOVEL H-NS PARALOGUE IDENTIFIED IN THE ENTEROAGGREGATIVE E. COLI STRAIN 042 HAS EVOLVED TO SPECIFICALLY MODULATE HGT DNA

A. Prieto[1], M. Bernabeu[1], S. Aznar[2], M. Huttener[1], A. Juárez[1]
[1]Universitat de Barcelona,
Departamento de Genética- Microbiología y Estadística. Facultad de Biología. Universidad de Barcelona- Barcelona- Spain, Barcelona, Spain
[2]Instituto de Bioingeniería de Cataluña IBEC- Barcelona- Spain.,
Microbial Biotechnology and Host Pathogen Interaction, Barcelona, Spain

Backgrounds

Bacterial nucleoid-associated proteins contribute to nucleoid structure and modulate gene expression. The H-NS protein is widespread in Gammaproteobacteria and has been best studied in *E. coli* and related genera. H-NS binds to DNA in a non-sequence-specific manner. H-NS binds, mainly HGT DNA which, among others, encodes virulence determinants. StpA is an H-NS paralogue which in *E. coli* appears not to have a specific modulatory role out of functioning as an H-NS molecular backup.

Objectives

A recent genomic analysis performed by our group has shown that, unlike many other *E. coli* strains, the chromosome of the strain 042 encodes a new paralogue of the *hns* gene, *hns2*. We present in this communication information about the biological role of this new member of the H-NS family of proteins.

Methods

We performed RNAseq experiments with a set of EAEC 042 mutants: *hns*, *hns2* and *hnshns2.* We compared then the gene expression pattern to the wild type strain 042. In addition, we measured H-NS2 expression by qPCR as well as by immunodetection.

Conclusions

H-NS2 expression is significantly lower than that of H-NS. H-NS2 modulates a subset of the H-NS modulated genes, mainly HGT genes. The obtained data suggest a specialized role for H-NS2 modulating HGT DNA. The observed low expression levels of H-NS2 together with the likely enhanced sensitivity to proteolysis suggest that enterobacterial strains expressing this regulator may specifically derepress HGT genes under stress conditions without altering other H-NS specific functions such as chromosome architecture or modulation of core genes.

FEMS7-1920
Physiology / Biochemistry / Molecular Microbiology - Part II

THE EFFECT OF PEROXISOME ON VARIOUS ENVIRONMENT BY CHANGE OF PEROXINS IN SACCHAROMYCES CEREVISIAE

H.J. Kang[1], B.H. Choi[1], P.C. Lee[1], H.J. Hwang[1]
[1]Ajou University, Department of Molecular Science and Technolgy, Woncheon-dong- Yeongtong-gu- Suwon, Republic of Korea

Backgrounds

In *S. cerevisiae*, the regulation of peroxisome, which has various metabolic processes, is tightly controlled by several peroxins depending on the environment. To date, 34 peroxins have been identified that are involved in the peroxisome biogenesis, proliferation and targeting and importing of peroxisomal matrix proteins. However, exact regulation mechanism of peroxin for number and size of peroxisome on various environment remain unclear.

Objectives

In this study, to determine the exact mechanism for the number and size regulation of peroxisome, we investigated the effect of *PEX34* and *PEX11*, which are known to be involved in the peroxisome population and size, on various environments in *S. cerevisiae*.

Methods

The CEN.PK2-1D *S. cerevisiae* strain and its derivatives were used in this study. To investigate changes for the number and size of peroxisomes in various environments, deletion or/and overexpression strains of peroxin gene were constructed. Morphology, size and abundance of peroxisome was observed by TEM (Transmission electron microscopy) and Pot1-GFP tagging or GFP-PTS1 (peroxisomal targeting signal 1) expression vector. RNA-Seq analysis was performed to determine the effect of changes on number and size of peroxisome in yeast cell.

Conclusions

In the present study, we confirmed the change in numbers and sizes of peroxisome by the regulation of *PEX34* and *PEX11*. Furthermore, it was observed that changes of peroxisome affected the stress response of yeast cells. The results of our study provide clues to understand the regulation mechanism for number and size of peroxisome by peroxins and may be useful for peroxisome utilization in metabolic engineering and synthetic biology.

FEMS7-2715
Physiology / Biochemistry / Molecular Microbiology - Part II

GLYCAN-CONJUGATED FLUORESCENT AND MAGNETIC NANOPARTICLES FOR DETECTION OF HELICOBACTER PYLORI

J.Y. Hyun[1]
[1]*Yonsei University, Chemistry, Seoul, Republic of Korea*

Backgrounds

Helicobacter pylori is known to be a causative pathogen to induce chronic gastritis and gastric ulcers. It is also linked to the development of duodenal ulcers and stomach cancer.

Objectives

As a consequence, a sensitive method to detect *H. pylori* is crucial for diagnosis and treatment of *H. pylori*-associated diseases. It was reported that some strains of *H. pylori* express sialic acid-binding (SabA) and Le^b-binding adhesins (BabA) that recognize glycans on the human gastric mucosa for infection.

Methods

To sensitively detect *H. pylori* strains expressing BabA, fluorescent magnetic glyconanoparticles were prepared by conjugating aminoethylated Le^a, Le^b and H1 oligosaccharides to carboxy-containing fluorescent magnetic nanoparticles. The glycoclusters were characterized by using FT-IR, TEM and DLS/zeta potential techniques. They were then utilized to detect *H. pylori*. In this study, *H. pylori* J99 strain which expresses BabA was incubated with three kinds of glyconanoparticles.

Conclusions

The results of fluorescence microscopy analysis showed that Le^b and H1 conjugated but not Le^a conjugated nanoparticles bound to this strain. However, these glyconanoparticles did not recognize *H. pylori* strains lacking BabA. They were also employed to enrich BabA expressing *H. pylori* by using a magnet. It is anticipated that dual-mode glyconanoparticles will be powerful tools to sensitively detect pathogens including *H. pylori*.

FEMS7-2716
Physiology / Biochemistry / Molecular Microbiology - Part II

THE CARBOHYDRATE MICROARRAY TECHNOLOGY IS A POWERFUL TOOL FOR SCREENING OF FUNCTIONAL GLYCANS

J.Y. Hyun[1], I. Shin[1]
[1]Yonsei University, Chemistry, Seoul, Republic of Korea

Backgrounds

It is well-recognized that carbohydrate-binding proteins (lectins) expressed on the cell surface are involved in a wealth of biological processes through interactions with free or conjugated glycans.

Objectives

For example, cell surface lectins in the immune system bind to microorganism glycans on the surface, an event which leads to stimulation of immune responses to microorganisms. Mouse SIGN-R1 (SIGN-related 1) is a homolog of human DC-SIGN (dendritic cell-specific ICAM-grabbing non-integrin) that is expressed largely on macrophages. This lectin recognizes mannose-rich or fucosylated glycans in a Ca^{2+}-dependent manner. When SIGN-R1 interacts with glycans expressed on microorganisms, glycan antigens are internalized into cells via lectin-mediated endocytosis to induce immune activation. Thus, it is of great importance to identify glycans that elicit immune cell response.

Methods

In this study, a carbohydrate microarray-based technology was utilized for the rapid screening of glycans that trigger production of reactive oxygen species (ROS) through binding to the cell-surface SIGN-R1. To identify functional glycans which enhance ROS production, a fluorescent ROS probe was employed. It was found that binding of SIGN-R1 expressing cells to several mannose and fucose containing glycans immobilized onto the microarrays generated ROS, whose levels were attenuated in the presence of a ROS scavenger or a NADPH oxidase inhibitor.

Conclusions

The findings indicate that glycan microarrays are applicable to the simultaneous screening of functional glycans whose binding to the cell-surface lectin elicits cellular response.

FEMS7-2711
Physiology / Biochemistry / Molecular Microbiology - Part II

EPIDEMIOLOGY OF EXTENDED-SPECTRUM BETA LACTAMASES AND CARBAPENEMASES IN KLEBSIELLA PNEUMONIAE HOSPITAL ISOLATES FROM MOSTAR, BOSNIA AND HERZEGOVINA

A. Ibrahimagić[1], F. Kamberović[1], T. Petrović[2], S. Jakovac[2], D. Slačanac[3], S. Uzunović[1], B. Bedenić[4], J. Luxner[5], I. Barišić[6]
[1]Institute for Health and Food Safety, Department for Laboratory Diagnostics, Zenica, Bosnia - Herzegovina
[2]University Clinical Hospital, Department for Microbiology and Molecular Microbiology, Mostar, Bosnia - Herzegovina
[3]University of Zagreb, School of Medicine, Zagreb, Croatia
[4]Clinical Hospital Center, Laboratory for Molecular Microbiology- Department for Clinical Microbiology, Zagreb, Croatia
[5]Institute for Microbiology, Hygene and Environmental Medicine, Gratz, Austria
[6]Institute for Technology, Institute for Technology, Vienna, Austria

Backgrounds

Extended-spectrum beta lactamase (ESBL) producing isolates has become one of the most challenging agents of nosocomial infections in recent years.

Objectives

To determine antibiotic susceptibility and to characterize ESBL/carbapenem-resistance of eleven *Klebsiella pneumoniae* isolates collected in hospital, Mostar, Bosnia and Herzegovina.

Methods

Eleven *Klebsiella pneumoniae* isolates with reduced susceptiblity to cephalosporins and carbapenems were isolated. Antibiotic susceptibility was determined by broth microdilution method. Production of metallo-β-lactamases (MBLs) and ESBLs was detected by inhibitor-based tests with EDTA and clavulanic acid, respectively. Genes encoding OXA-23/OXA-24/40-/OXA-58-/OXA-143-like carbapenem-hydrolzing oxacillinases, in addition to MBLs of VIM, IMP and SIM series, SHV, CTX-M, PER-1 and TEM β-lactamases were detected by PCR.

Conclusions

All isolates were found to be multidrug resistant and ESBL producers. Cefuroxime, ceftazidime, cefotaxime and ceftriaxone were the least potent antibiotics with MIC_{90} >128 mg/L, cefepime MIC_{90} 64 mg/L, gentamicine MIC_{90} >128 mg/L and ciprofloxacin MIC_{90} 64 mg/L. Conjugation frequency was in the range 10^{-3}–10^{-5}. Resistance to gentamicin, sulphamethoxazole, and tetracycline in most cases were co transferred alongside with cefotaxime resistance. MBL fenotype test in one isolate was positive, but the PCR targeting common MBLs was negative. All strains were positive for TEM and CTX-M genes. Antimicrobial susceptibility testing and molecular characteristics of *K. pneumoniae* isolates showed a significantly high level and prevalence of resistance and high prevalence of both CTX-M and TEM genes. This is the first report of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases in Mostar, Bosnia and Hercegovina.

FEMS7-1564
Physiology / Biochemistry / Molecular Microbiology - Part II

BORONATE CARRYING NANOPARTICLES FOR THE PURIFICATION OF RNA FROM ESCHERICHIA COLI

N. Idil[1], *I. Perçin*[1], *A. Denizli*[2]
[1]*Hacettepe University, Department of Biology, Ankara, Turkey*
[2]*Hacettepe University, Department of Chemistry, Ankara, Turkey*

Backgrounds

Boronate affinity chromatography has been exploited for the separation of a wide variety of cis-diol-containing compounds including RNA. In recent years, boronate affinity functionalized materials have attracted great interest in nucleic acid-based researches due to being effective sorbents. Nanoparticles have widespread use in many applications due to their unique chemical and physical properties and they are capable of reducing the purification time.

Objectives

The objectives of the study were to prepare boronate carrying nanoparticles in order to purify RNA from *Escherichia coli* and investigate RNA binding capacity of these nanoparticles at different experimental conditions.

Methods

Poly(hydroxyethyl methacrylate-co-vinylphenylboronic acid) [P(HEMA-VPBA)] nanoparticles were prepared for the purification of RNA from *Escherichia coli.* These nanoparticles were synthesized using vinylphenylboronic acid (VPBA) and 2- hydroxyethyl methacrylate (HEMA) via miniemulsion method. The RNA binding capacity of [P(HEMA-VPBA)] nanoparticles were examined in a batch system.

Conclusions

The prepared nanoparticles were selected as a boronic acid affinity system for the purification of RNA from *Escherichia coli.* In this manner, it was determined that [P(HEMA-VPBA)] nanoparticles had high efficiency when purifying RNA from a biological source. It can be concluded that these boronic acid carrying nanoparticles were capable of separate the bacterial RNA successfully and promising tools for the purification of RNA.

FEMS7-2440
Physiology / Biochemistry / Molecular Microbiology - Part II

BORONATE AFFINITY NANOPARTICLES FOR IGG BINDING

N. Idil[1], I. Perçin[2], A. Denizli[3]
[1]Hacettepe University, Department of Biology- Biotechnology, Ankara, Turkey
[2]Hacettepe University, Department of Biology, Ankara, Turkey
[3]Hacettepe University, Department of Chemistry, Ankara, Turkey

Backgrounds

Immunoglobulins are glycoproteins which have crucial roles in immune system response. There have been many separation techniques for IgG including affinity chromatography. Boronate affinity adsorbents have been widely used for the separation of biomolecules such as glycoproteins by selective capturing of cis-diols. Nanosized particles provides high-capacity boronate affinity adsorbents with high surface areas.

Objectives

The objectives of the study were (i) to prepare boronate affinity nanoparticles for IgG binding, (ii) to characterize these nanoparticles by particle size distribution, Fourier transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM), (iii) to investigate IgG binding capacity of prepared nanoparticles at different experimental conditions.

Methods

The phenylboronic acid (PBA) was preferred as a ligand due to the specific and reversible interaction with glycoproteins. Poly(hydroxyethyl methacrylate-co-vinylphenylboronic acid) [P(HEMA-VPBA)] nanoparticles were synthesized for IgG binding.

Conclusions

The proposed [P(HEMA-VPBA)] nanoparticles have the ability to bind IgG and they are promising candidates to be used in many biotechnological applications offering the advantage of high binding capacity.

FEMS7-0314
Physiology / Biochemistry / Molecular Microbiology - Part II

TRANSCRIPTOMIC ANALYSIS ON THE RESPONSE OF PORCINE ADIPOCYTES TO COMMON MICROBIAL LIGANDS

M. Igata[1], A. Tada[1], A.H. Kober[1], L. Albarracín[2], W. Ikeda-Ohtsubo[1], H. Aso[1], K. Miyazawa[3], K. Yoda[3], F. He[3], T. Saito[1], J. Villena[2], H. Kitazawa[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan
[2]CENTRO DE REFERENCIA PARA LACTOBACILOS CERELA-CONICET, Laboratory of Immunobiotechnology, San Miguel de Tucumán, Argentina
[3]Takanashi Milk Products Co.- Ltd, Technical Research Laboratory, Yokohama, Japan

Backgrounds

Previous we established a clonal porcine intramuscular preadipocyte cell line and a protocol to obtain functional porcine mature adipocytes (PMAs) from these cells.

Objectives

In this work, we investigated the immunobiology of PMAs in relation to their response to Toll-like receptor 2 (TLR2) or TLR4 stimulation by using a transcriptomic approach.

Methods

PMAs cells were stimulated with peptidoglycan or lipopolysaccharide (LPS), and the transcriptomic response was evaluated 12 h after the challenge using the Porcine (V2) Gene Expression Microarray (Agilent). Variations in transcript abundance with a t-test $p<0.05$, and a cutoff in abundance of at least 2-fold were considered statistically different. Changes in the expression of selected genes were confirmed by Real-Time RT-PCR.

Conclusions

Both, TLR2 and TLR4 activation increased the expression of inflammatory factors in PMAs including chemokines (*CCL2, CCL8, CXCL2*), adhesion molecules (*VCAM1*) and complement factors. However, the immunomodulatory effect was stronger in TLR2-stimulated PMAs than in those receiving LPS, since increased expression of *IL-1, CCL20, CXCL12, EPCAM, SELL*, and *NOS2* was found only in the first group. On the contrary, TLR4 activation had a stronger influence in lipid metabolism. Genes involved in lipid metabolism such as *APOB, APOM, FFAR2, MGPD*, and *PLA2G7* were up-regulated in LPS-treated PMAs but not in those stimulated with peptidoglycan. These results indicate that PMAs are able to generate complex metabolic/immune responses to common microbial ligands. Therefore, PMAs could be used for the *in vitro* screening and selection of new alternatives to beneficially modulate adipose tissue metabolism and/or inflammation such as probiotic treatments.

FEMS7-1659
Physiology / Biochemistry / Molecular Microbiology - Part II

POPULATION STRUCTURE IN CAMPYLOBACTER JEJUNI ISOLATED FROM HUMANS, CHICKENS AND WILD BIRDS

Y. Iglesias[1], *E. Miro*[1], *C. Muñoz*[1], *T. Llovet*[1], *C. Madrid*[2], *C. Balsalobre*[2], *F. Navarro*[1]
[1]*Hospital de la Santa Creu i Sant Pau, Microbiology, Barcelona, Spain*
[2]*Universidad de Barcelona, Microbiology, Barcelona, Spain*

Backgrounds

Campylobacter jejuni is responsible for most foodborne bacterial infections worldwide. It is believed that the major source of contamination is caused by consumption of contaminated chicken meat.

Objectives

The objectives of this study were to compare the clonal diversity and population structure between *C. jejuni* strains from human, chickens and wild birds.

Methods

A total of 150 *C. jejuni* strains were analyzed, 50 from patients attended at Hospital de Sant Pau in 2014, 50 from broiler isolated from different farms in 2013 and 50 strains from five different species of wild birds obtained between 2014 and 2015. Identification at the species level was performed by MALDI-TOF. The population study was carried out by PFGE with *Sma*I and *Kpn*I, and MLST (http://Pubmlst.org/ campylobacter).

Conclusions

Among the 150 strains 60 ST were obtained (six of them for the first time in this study) grouped into 21 clonal complexes (CC) and 12 singletons (S). ST-45, ST-48 and ST-354 were present in the three collections. The CC-21, CC-1275, CC-45 and CC-257 were the most prevalent. By PFGE 143 restriction patterns were obtained. Only three clusters with more than one strain were obtained. One of them included six strains from chicken, whereas the other two clusters include two strains of wild bird each one. There is great genotype variability between the three collections. Analysis data using both methods classify clearly separates the bird population of human and chicken. Human and chicken population shared ST profiles but no PFGE .

FEMS7-0960
Physiology / Biochemistry / Molecular Microbiology - Part II

INSIGHTS INTO THE ROLE OF FLUG IN THE REGULATION OF DEVELOPMENT IN ASPERGILLUS

M. Iradi[1], *M. S. Cortese*[1], *U. Ugalde*[1]
[1]*University of the Basque Country, Applied Chemistry, San Sebastian, Spain*

Backgrounds

FluG was recognized as an early regulator of asexual development over two decades ago. *fluG* null mutants show profuse aerial growth and no conidial production in minimal medium, but can display moderate sporulation under nitrogen limitation. Initial studies detected sequence homology of *fluG* with a prokaryotic glutamine synthetase (GS), but catalytic activity was not demonstrated. Other investigations placed FluG as a putative interactor of the Velvet complex.

Objectives

1) To perform a detailed and updated bioinformatic analysis of FluG sequence.
2) Make point mutations in predicted catalytic residues to confirm the functionalities of the predicted protein.

Methods

We conducted a sequence and crystal structure analysis of the FluG sequence, which yielded two clearly distinguishable regions: a N-terminal amidohydrolase (AH) coding region and a C-terminal GS or gamma-glutamyl synthetase region. Separate expression of these regions revealed that the C-terminal region was essential for asexual development. The N-terminal region, in turn, was required only under high nitrogen availability.
Selected point mutations directed at disabling catalytic residues with no major predictable structural changes caused severe developmental defects.

Conclusions

We have therefore concluded that FluG may act as a two-enzyme multifunctional protein. Given that intracellular glutamine levels are associated with growth of aerial hyphae and suppression of asexual development (fluffy phenotype), we hypothesize that FluG may scavenge nitrogen intermediates which could be otherwise destined for glutamine synthesis, thus preventing the formation of aerial hyphae. The product of the FluG enzyme would, instead, signal or metabolically channel the induction of asexual development.

FEMS7-2772
Physiology / Biochemistry / Molecular Microbiology - Part II

GRAM NEGATIVE PROMISCUOUS LIPOPROTEINS KEEP SURFACE TOPOLOGY WHEN TRANSPLANTED FROM ONE SPECIES TO ANOTHER AND CAN DELIVER FOREIGN POLYPEPTIDES TO THE BACTERIAL SURFACE

C. Irene[1], L. Fantappie'[1], M. De Santis[1], A. Armini[2], A. Gagliardi[2], M. Tomasi[1], M. Parri[3], V. Cafardi[1], S. Bonomi[3], L. Ganfini[1], F. Zerbini[1], I. Zanella[1], C. Carnemolla[2], L. Bini[2], A. Grandi[3], G. Grandi[1]
[1]University of Trento, CIBIO, Trento, Italy
[2]University of Siena, Department of Life Sciences, Siena, Italy
[3]Toscana Life Science, Toscana Life Science, Siena, Italy

Backgrounds

In Gram negative bacteria outer membrane-associated lipoproteins can either face the periplasm or protrude out of the bacterial surface. The mechanisms involved in lipoprotein transport through the outer membrane are not fully elucidated. A group of lipoproteins reach the surface by using species-specific transport machineries. By contrast, a still poorly characterized group, we referred to as “promiscuous lipoproteins”, appears to cross the outer membrane using transport systems shared by different species.

Objectives

To provide further evidence of the existence of promiscuous lipoproteins, we tested the expression and compartmentalization in *E. coli* of three surface-exposed lipoproteins, two from *Neisseria meningitidis* (Nm-fHbp and NHBA) and one from *Aggregatibacter actinomycetemcomitans* (Aa-fHbp). We found that the three lipoproteins were lipidated and compartmentalized in *E. coli* outer membrane and in Outer Membrane Vesicles (OMVs). Furthermore, FACS analysis, proteolytic surface shaving, and confocal microscopy revealed that the three proteins were also exposed on the external side of the outer membrane. Removal or substitution of the first four amino acids following the lipidated Cysteine residue and extensive deletions of the C-terminal regions in Nm-fHbp did not prevent the protein from reaching the external side of the outer membrane. Heterologous polypeptides fused to the C termini of the proteins are efficiently transported to the *E. coli* surface and efficiently compartmentalized in OMVs.

Methods

Outer membrane vesicles preparation by ultracentrifugation, Triton-X114 phase fractionation, SDS-PAGE, FACS analysis, proteolytic surface shaving, confocal microscopy, Two-dimentional gel electrophoresis, protein identification by mass spectrometry

Conclusions

These data indicate that promiscuous lipoproteins travel from the cytoplasm to the cell surface using conserved mechanisms and when transplanted from one species to another they maintain their natural topology without the need of ancillary transport machineries. Furthermore such proteins can be exploited in biotechnological applications requiring the decoration of Gram negative bacterial surface with foreign polypeptides.

FEMS7-2010
Physiology / Biochemistry / Molecular Microbiology - Part II

QUANTIFICATION OF CELL ADHESION OF ACINETOBACTER SP. TOL 5 VIA A TRIMERIC AUTOTRANSPORTER ADHESIN USING ATOMIC FORCE MICROSCOPY

S. Ishii[1], S. Yoshimoto[1], K. Hori[1]
[1]Nagoya University, engineering, Nagoya, Japan

Backgrounds

The bacterial adhesion to material surfaces or host cells is mediated by cell surface proteins called adhesins. Trimeric autotransporter adhesin (TAA) is one of the major non-fimbrial adhesins widely distributing in Gram-negative bacteria. *Acinetobacter* sp. Tol 5 shows autoagglutinating nature and noteworthy adhesiveness through its TAA, namely AtaA. We previously reported the unique adhesiveness of Tol 5 cells to various abiotic material surfaces from hydrophobic plastics to hydrophilic glass and metals. However, the adhesion force of Tol 5 cells via AtaA has only preliminary been investigated.

Objectives

In this work, we evaluated the strength and properties of adhesion of Tol 5 cells via AtaA using atomic force microscopy (AFM). We also attempted to quantify adhesion force of a single AtaA molecule.

Methods

We measured the interaction force between a cantilever probe and a cell surface of various bacteria including Tol 5, *Pseudomonas aeruginosa*, *Escherichia coli and Yersinia enterocolitica* in liquid. These cells were immobilized on the polyethyleneimine-modified glass plate by using aldehyde-cross-linkage before the measurement.

Conclusions

Tol 5 cells showed much stronger adhesion force than the D*ataA* mutant of Tol 5 and other typical bacteria did. The result revealed that AtaA confers not only high adhesiveness but also strong adhesion force to Tol 5 cells.

FEMS7-2280
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERIZATION OF AN EPSILON/ZETA TOXIN-ANTITOXIN SYSTEM FROM THE CLINICAL STRAIN MYCOBACTERIUM SP. MHSD3, CLOSELY RELATED TO MYCOBACTERIUM CHELONAE.

D. Jaén-Luchoro[1], F. Aliaga-Lozano[2], R.M. Gomila[3], M. Gomila[4], F. Salvà-Serra[5], J. Lalucat[4], A. Bennasar-Figueras[4]
[1]University of the Balearic Islands, Biology, Palma de Mallorca, Spain
[2]Clínica Rotger, Laboratoy of Molecular Biology, Palma de Mallorca, Spain
[3]University of Balearic Islands, Serveis Cientifico-Tècnics-, Palma de Mallorca, Spain
[4]University of Balearic Islands, Biology, Palma de Mallorca, Spain
[5]University of Gothenburg, Infectious desease, Gothenburg, Sweden

Background

Mycobacterium chelonae, an emergent opportunistic pathogen, causes infections difficult to treat mainly due to its high antibiotic resistance, being necessary to find alternative targets for new treatments to fight against them. Toxin-antitoxin systems (TAS), small operons that can contribute to the virulence of the strain, are considered important potential targets.

Objectives

The main objectives are i) to identify the potential toxin-antitoxin systems (TAS) in the clinical strain Mycobacterium sp. MHSD3, and ii) to characterize the TAS from a genomic, functional and structural approach.

Methods

BLAST against TADB and GenBank databases were made to find potential TAS and possible homologues, respectively. Functional domains were searched in Pfam database. Antitoxinsand toxins were clonedseparately into expresion vectors: pBAD/HA (inducible with arabinose) and pRSF-DUET (inducible with IPTG), respectively. Constructions were transformed in Escherichia coli BL21 (DE3) pLysS and cultured on LB agar plates supplemented with kanamicin (pRSF-Duet), ampicilin (pBAD/HA) and cloramphenicol (pLysS). Positive clones were used for functional assays in four different conditions: control (no induction), antitoxin induction (arabinose),toxin induction (IPTG) and induction of both (arabinose+IPTG). Cells were grown to the stationary phase, maintaining the antibiotic presure. Absorbance at 600 nm and colony counting mesurements were made. Expresion of the protein was confirmed by MALDI-TOF MS analysis. Structure prediction was performed using I-TASSER.

Conclusion

A functional type II epsilon/zeta toxin-antitoxinsystem was found in Mycobacterium sp. MHSD3. The system was found close to genes codifying for proteins containing β-lactamases andintegrases/transposases domains, representing a potential mobile genetic element stabilizing antibiotic resistance genes.

FEMS7-1453
Physiology / Biochemistry / Molecular Microbiology - Part II

UNVEILING THE HYBRID GENOME STRUCTURE OF ESCHERICHIA COLI RR1 (HB101 RECA+)

H. Jeong[1], Y.M. Sim[2], H.J. Kim[3], S.J. Lee[3]
[1]Korea Research Institute of Bioscience and Biotechnology, Infectious Disease Research Center, Daejeon, Republic of Korea
[2]Korea Research Institute of Bioscience and Biotechnology, Korean Bioinforation Center, Daejeon, Republic of Korea
[3]Korea Research Institute of Bioscience and Biotechnology, Metabolic Regulation Research Center, Daejeon, Republic of Korea

Backgrounds

There have been extensive genome sequencing studies for *Escherichia coli* strains, particularly for pathogenic isolates. For laboratory *E. coli* strains, however, genome sequence information is limited except for several well-known strains.

Objectives

The availability of accurate genome information for each strain is crucial to the success of a particular application. In this study, we determined the complete genome sequence of *Escherichia coli* RR1 (a *recA*+ derivative of HB101), a host suitable as a multipuprose cloning host.

Methods

A hybrid genome sequence of K-12 MG1655 and B BL21(DE3) was constructed based on the initial mapping of Illumina HiSeq reads to each reference, and iterative rounds of read mapping, variant detection, and consensus extraction were carried out. Finally, PCR and Sanger sequencing-based finishing were applied to resolve non-single nucleotide variant regions with aberrant read depths and breakpoints, most of them resulting from prophages and insertion sequence transpositions that are not present in the reference genome sequence.

Conclusions

We found that 96.9% of the RR1 genome is derived from K-12, and identified exact crossover junctions between K-12 and B genomic fragments. However, because RR1 has experienced a series of genetic manipulations since branching from the common ancestor, it has a set of mutations different from those found in K-12 MG1655. As well as identifying all known genotypes of RR1 on the basis of genomic context, we found new mutations. Our results extend current knowledge of the genotype of RR1 and its relatives, and provide insights into the pedigree, genomic background, and physiology of common laboratory strains.

FEMS7-2671
Physiology / Biochemistry / Molecular Microbiology - Part II

WHOLE GENOME SEQUENCING INSIGHTS INTO THE POPULATION BIOLOGY AND SECONDARY METABOLITE CAPACITY OF PATHOGENIC BURKHOLDERIA GLADIOLI

C. Jones[1], M. Bull[1], M. Jenner[2], L. Song[2], Y. Dashti[2], G. Webster[1], T. Spilker[3], S.R. Harris[4], J. Parkill[4], T.R. Connor[1], G.L. Challis[2], J.J. LiPuma[3], M. Eshwar[1]
[1]Cardiff University, School of Biosciences, Cardiff, United Kingdom
[2]University of Warwick, Department of Chemistry, Warwick, United Kingdom
[3]University of Michigan Medical School, Department of Pediatrics, Ann Arbor, USA
[4]Welcome Trsut Genome Campus, Welcome Trust Sanger Institute, Cambridge, United Kingdom

Backgrounds

The *Burkholderia* genus encompasses diverse and versatile species, infection with which is associated with poor clinical outcome in persons with cystic fibrosis (CF). One such pathogen, *Burkholderia gladioli*, was responsible for between 15 – 18 % of *Burkholderia* CF infections annually between 1997 and 2016, and is also a pathogen of commercially important crops.

Objectives

The genome sequences of *B. gladioli* isolates from CF and environmental sources were sequenced in order for population biology and secondary metabolism to be investigated.

Methods

Draft genome sequences of over 200 *B. gladioli* isolates were assembled and their secondary metabolite capacity analysed using AntiSMASH. Core genome phylogeny was used to compare genomes.

Conclusions

A wealth of biosynthetic loci were identified, with around 18% of genomic capacity devoted to the production of secondary metabolites and between 15 and 25 biosynthetic gene clusters (BGC's) per isolates. Very few BGC's were found in all isolates, and the total biosynthetic potential of individual isolates was variable. Analysis of bioactive fractions yielded multiple known (e.g. Enacyloxin, Bongkrekic acid, and Toxoflavin) and novel secondary metabolites.

The population biology of this diverse species was brought up to date. Our genomic comparison of environmental and CF isolates confirms that there is no clear CF lineage. Mapping the distribution of biosynthetic loci against the core genome phylogeny has provided new insights into the evolution of *B. gladioli*. By combining genome mining and population biology we aim to exploit the secondary metabolite potential of *B. gladioli*, for the production of novel secondary metabolites.

FEMS7-0939
Physiology / Biochemistry / Molecular Microbiology - Part II

COMPLETE GENOME SEQUENCE ANALYSIS OF PAENIBACILLUS KONKUKENSIS ISOLATED FROM PIG CONCENTRATE FEED

H.I. Jung[1], K. Yi[1], K. Niu[1], S.W. Lee[2], S.K. Kim[1]
[1]Konkuk University, Department of Animal Sciences and Technology, Seoul, Republic of Korea
[2]Konkuk University, College of Veterinary Medicine, Seoul, Republic of Korea

Backgrounds

Paenibacillus is a genus of Gram-positive, facultative anaerobic rod-shaped, and endospore-forming bacteria. *Paenibacillus* spp. have been detected in divergent environments such as soil, water, rhizosphere, vegetable matter, foods, tree roots, forage, and insect larvae. The main characteristics of *Paenibacillus* spp. is secretion of extracellular enzymes in neutral and alkaline growth conditions and production of polysaccharides, amino acids, and secondary metabolites such as antimicrobial agents, pigments, digestive enzymes, and growth promoters for vegetable and animal.

Objectives

In this study, we have completely sequenced for whole genome of a novel species *Paenibacillus konkukensis* SK3146 from pig concentrate feed and analyzed for beneficial genes as feed additive enzymes.

Methods

Complete genome sequencing of *P. konkukensis* SK3146 was performed using the PacBio® RS II system. Sequencing reads were *de novo* assembled using the PacBio SMRT analysis with default options. Annotation of open reading frames and functional gene analysis were carried out using the Prokka and the BASys softwares, respectively.

Conclusions

P. konkukensis SK3146 has a single circular form of genome that is 7,968,964 bp in size with 53.4% of GC contents. Presence of virulence factors, prophages, and the genes related in the CRISPER-Cas system and antibiotics resistance in the genome were analyzed. The genome encodes functional genes involved in various hydrolytic enzymes such as α-galactosidase, β-glucosidase, β-xylosidase, cellulase, lipase, protease, and xylanase etc. The results suggest that P. konkukensis SK3146 would be a valuable probiotic strain, which secrets variable digestive enzymes and could be used as feed additives in the animal industry.

FEMS7-0758
Physiology / Biochemistry / Molecular Microbiology - Part II

A NOVEL MECHANISM OF INHIBITION OF TRANSLATION INITIATION PRESENTED BY ACETYLTRANSFERASE TOXIN FROM TA MODULE

D. Jurenas[1], S. Chatterjee[2], A. Konijnenberg[2], F. Sobott[2], L. Droogmans[3], A. Garcia-Pino[4], L. Van Melderen[1]
[1]Free University of Brussels ULB, Laboratoire de Génétique et Physiologie Bactérienne, Gosselies, Belgium
[2]University of Antwerp, Center for Proteomics, Antwerp, Belgium
[3]Free University of Brussels ULB, Laboratoire de Microbiologie, Gosselies, Belgium
[4]Free University of Brussels ULB, Laboratoire de Biologie Structurale et Biophysique, Gosselies, Belgium

Backgrounds

Toxin-antitoxin (TA) modules are small operons widely spread in bacteria. TA modules are believed to be involved in stress response and bacterial persistence. Toxins from type II TAs most commonly are translation inhibitors that act at different stages of translation. Their targets span mRNAs, tRNAs and their charging, rRNAs and translation factors. Small proteins possessing acetyltransferase domain from GNAT family were found to constitute active TA pairs in several pathogenic bacteria.

Objectives

We aimed to characterize at the molecular level the toxicity mechanism of AtaT toxin involved in TA system AtaR-AtaT from enteropathogenic *E. coli* O157:H7.

Methods

Translation was followed *in vivo* and *in vitro* to detect the target of AtaT toxin. Acetylated target and precise modification position was identified by *in vitro* acetylation reactions with isotope labelled acetyl-CoenzymeA and by mass spectrometry. We have further *in vitro* reconstructed the translation initiation events by incorporation of isotopes and by ITC, as well as assayed changes in ribosome profiles *in vivo*.

Conclusions

We have found that AtaT acetylates the free amine group of methionine charged on initiator Met-$tRNA^{fMet}$. Acetyl-Met-$tRNA^{fMet}$ fails to interact with initiation factor-2 thereby hindering 30S initiation complex formation and therefore leading to translation initiation inhibition. High specificity of AtaT for the Met-$tRNA^{fMet}$ in comparison to other charged tRNAs leads to efficient inhibition of translation initiation which is manifested by accumulation of ribosome assembly intermediates.

FEMS7-1675
Physiology / Biochemistry / Molecular Microbiology - Part II

TOXIN-ANTITOXIN SYSTEM UNDER LAMBDOID PROPHAGE REGULATION

D. Jurenas[1], A. Vandervelde[2], P. De Bruyn[2], T. Jové[3], R. Loris[2], L. Van Melderen[1]
[1]Free University of Brussels ULB, Laboratoire de Génétique et Physiologie Bactérienne, Gosselies, Belgium
[2]Vrije Universiteit Brussel, Structural Biology Brussels- Department of Biotechnology, Brussels, Belgium
[3]Université de Limoges, INSERM- CHU Limoges, Limoges, France

Backgrounds

Bacterial Toxin-antitoxin (TA) modules of type II are typically small operons coding for two proteins: a bacterial cell growth inhibitor and its cognate antidote that binds toxin to form an inactive complex. Antitoxins usually present a DNA-binding domain and are also responsible for regulation of transcription of the TA operon. In several cases TAs are found in three component configurations where regulator and antitoxin are two separate proteins. PaaR2-PaaA2-ParE2 (RAE2) module from enteropathogenic *E. coli* O157:H7 is located in a lambdoid prophage region which provides possibility of complex regulation.

Objectives

We aimed to investigate the transcriptional regulation of *E. coli* O157:H7 CP993-P PaaR2-PaaA2-ParE2 system.

Methods

We used 5'RACE and N-terminal sequencing to detect transcription and translation start sites respectively. Promoter transcriptional activity cloned upstream of GFP was measured upon expression of different regulators. EMSA and DNA footprint assays were performed to confirm binding sites of regulators.

Conclusions

PaaR2 regulator shares several features with lambda cI regulator – localization, leaderless translation, multimerization, binding to additional promoter downstream of RAE2, but has different binding consensus than cI. YdaS-YdaT operon encoded upstream of RAE2 is implicated in transcriptional regulation of RAE2 with YdaS being a negative regulator and YdaT being a positive regulator of an alternative promoter for RAE2 located between YdaS and YdaT. Taken together our results provide an example of a TA module hijacked by a sophisticated transcriptional regulation of lambdoid prophage.

FEMS7-1861
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERIZATION OF THE ROLE OF SPA33, A COMPONENT OF THE TYPE 3 SECRETION SYSTEM IN SHIGELLA FLEXNERI

M. Kadari[1], D. Lakhloufi[1], H. Koroglu[1], P. SMEESTERS[1], A. Botteaux[1]
[1]Université libre de Bruxelles ULB, Laboratoire de Bactériologie moléculaire, Brussels, Belgium

Backgrounds

Shigella uses a surface nano-syringe, called type 3 secretion system (T3SS), to enter enterocytes. Upon cell contact, T3 secreted proteins, called translocators, form a pore inside the host cell membrane allowing the secretion of proteins, called the effectors, directly into the cell cytoplasm where they interfere with multiple signalling cascades. Nevertheless, the mechanism underlying this T3SS activation is still poorly understood.

Objectives

We aimed to characterize the role of a T3S cytoplasmic component, Spa33, in the secretion activation

Methods

The role of Spa33 was studied by generating point mutations and analyzed their impact on T3S. Binding partners of Spa33 were also investigated by GST pull-down assays.

Conclusions

We have identified an alternative translation initiation site inside the *spa33* gene leading to the expression of a short C-terminal fragment ($Spa33^C$). The suppression of $Spa33^C$ expression totally impairs the proteins secretion by the T3SS. Nevertheless, the ectopic expression of $Spa33^C$ *in trans* restores the secretion of the translocators, but not the one of effectors. The phenotype was shown to be a consequence of the secretion defect of one of the regulator, MxiC. Interestingly, we have shown that MxiC interacts with $Spa33^C$ strengthening our model in which MxiC, in concert with Spa33, prevent early secretion of T3 effectors.

FEMS7-2591
Physiology / Biochemistry / Molecular Microbiology - Part II

ENDOCYTIC PATHWAYS IN PLANT PATHOGENIC FUNGUS RHIZOCTONIA SOLANI

O.V. Kamzolkina[1], M.A. Kiselica[2], N.A. Krupenina[3], O.A. Kudryavtseva[2], O.V. Shtaer[2], I.S. Mazheika[2]
[1]Lomonosov MSU, Faculty of Biological- department of Mycology and Algology, Moscow, Russia
[2]Lomonosov MSU, Faculty of Biology- department of Mycology and Algology, Moscow, Russia
[3]Lomonosov MSU, Faculty of Biology- department of Biophysics, Moscow, Russia

Backgrounds

Endocytosis is a complex process of transport into the cell and further distribution of soluble substances and macromolecules with the participation of the plasma membrane forming vesicles. Nowadays it is described about ten different types of endocytosis in animals according to the differences in the initial stages. Endocytosis in filamentous fungi is not studied enough. Investigation of endocytic mechanisms in fungi of such kind is a relevant and promising area for fungal cell biology, fundamental and applied mycology.

Objectives

Inhibitory analysis of endocytosis dynamics in plant pathogenic fungus *Rhizoctonia solani.*

Methods

We used methods of tagging endocytic membrane with a fluorescent AM4-64.

Conclusions

We demonstrated that: a) actin cytoskeleton plays a key role in endocytosis; b) microtubular cytoskeleton plays an unusual role in endocytosis. It affects formation of primary vesicles that has not been shown for other organisms; c) inhibitors of different types of endocytosis (clathrin-dependent, dynamin-dependent and lipid-blast) decelerate or completely block it. Our results indicate the existence of conservative endocytic mechanisms in *R. solani* as well as in other eukaryotes. On the other hand, some features of endocytosis in *R. solani* suggest the presence of specific endocytic mechanisms in filamentous fungi.This work was supported by the Russian Foundation for Basic Research (RFBR) project № 16-04-00814.

FEMS7-1583
Physiology / Biochemistry / Molecular Microbiology - Part II

THE SMALL RNA CHAPERONE HFQ REGULATES QUORUM SENSING AND PATHOGENICITY OF PANTOEA ANANATIS

B. Kang[1], O. Choi[2], J. Park[1], Y. Lee[1], J. Kim[3]
[1]Gyeongsang National University, Division of Applied Life Science BK21 Plus, Jinju, Republic of Korea
[2]Gyeongsang National University, Institute of Agriculture & Life Science, Jinju, Republic of Korea
[3]Gyeongsang National University, Institute of Agriculture & Life Science- Division of Applied Life Science BK21 Plus, Jinju, Republic of Korea

Backgrounds

Hfq is an abundant bacterial RNA binding protein which has many important physiological roles that are usually mediated by interacting with Hfq binding small RNA (sRNA). *Pantoea ananatis* PA13, our model bacterium, causes bacterial rice sheath rot and onion center rot diseases.

Objectives

The objective of this study was to assess the contribution of the sRNA chaperone Hfq of *P. ananatis* to quorum sensing and pathogenicity.

Methods

Several phenotypes of Δ*hfq* mutant including carotenoid pigmentation, swimming motility, EPS production, HR in tobacco leaves, and virulence in rice and onion were assessed.

Conclusions

Δ*hfq* mutant exhibited the deficiency in carotenoid pigmentation, swimming motility, EPS production, HR in tobacco leaves and virulence in rice and onion. All phenotype deficiencies of Δ*hfq* mutant were recovered by genetic complementation with intact *hfq*(pCOK335; pLAFR3::*hfq*). A screen for additional circuits of the *Pantoea ananatis* QS revealed the protein Hfq. We showed that Hfq regulates the transcription of *eanI*(QS signal synthetic gene) and QS signal production. A small regulatory RNA (sRNA) SraH is known to bind Hfq and increase translation of sigma factor RpoS. Δ*sraH* mutant of *P. ananatis* exhibited deficiencies in EPS production, HR and virulence in rice and onion. All phenotypes deficiencies of Δ*sraH* mutant were recovered by genetic complementation with intact *sraH*(pBS28; pLAFR3::*sraH*), indicating SraH controls pathogenicity of PA13.

FEMS7-0220
Physiology / Biochemistry / Molecular Microbiology - Part II

IDENTIFICATION, BIOSYNTHESIS AND CHARACTERIZATION OF A NOVEL BACTERIOCIN ENCODED IN THE GENOME OF THERMOPHILIC BACTERIA AERIBACILLUS PALLIDUS

A. Kaunietis[1], *A. de Jong*[2], *A. Buivydas*[2], *O. Kuipers*[2]
[1]*Vilnius University, Life Sciences Center, Vilnius, Lithuania*
[2]*University of Groningen, Molecular Genetics, Groningen, The Netherlands*

Backgrounds

Bacteriocins are ribosomally synthesised antimicrobial peptides. Part of them can undergo post-translational modifications. It is a heterogeneous group of small (<10 kDa) peptides that kills other bacteria. They can have narrow or broad range of antibacterial activity spectrum. Producer strain has immunity mechanism to its own synthesised bacteriocin. The current increase of antibiotic resistance among bacteria requires new alternatives to traditional antibiotics. Bacteriocins have many properties like high activity, stability or low toxicity which suggest them as viable alternative. They can be used not only in treatment of human pathogens but also in food-safety applications.

Objectives

The aim of this study was to find genes coding new bacteriocins in the genome of thermophilic bacteria *Aeribacillus pallidus*.

Methods

Genomic DNA of bacteria was sequenced (NGS) on Illumina platform. Velvet algorithms set was used for *de novo* paired-end assembly, resulting draft genome sequence. The RAST, BAGEL3 and antiSMASH servers were used to annotate the genome and to identify putative gene clusters coding bacteriocins which subsequently were cloned to vectors and overexpressed in heterologous host *E. coli*. One of the synthesised antimicrobial peptide was purified using MPLC and HPLC systems and analysed in mass spectrometry methods.

Conclusions

BAGEL3 and antiSMASH observed that genome of *Aeribacillus pallidus* codes several gene clusters of putative biosynthetic machineries of antimicrobial compounds. One of these gene clusters was successfully overexpressed in *E. coli* and a novel active bacteriocin with post-translational modifications was synthesised.

FEMS7-0034
Physiology / Biochemistry / Molecular Microbiology - Part II

TRANSFER OF PLASMIDS FROM ESCHERICHIA COLI TO CLOSTRIDIUM DIFFICILE IS SENSITIVE TO DNASE.

L. Khodadoost[1], *P. Mullany*[1]
[1]*Eastman Dental Institute- University College London, Microbial Diseases, London, United Kingdom*

Backgrounds

Background

Horizontal gene transfer (HGT) between bacterial cells is an important mediator of bacterial evolution and adaption to various environments. In the clinical setting it is a major player in the spread of antibiotic resistance. Three mechanisms of HGT in bacteria are known: conjugation, transduction and transformation. HGT is also used to transfer genetic constructs made in organisms that have relatively advanced genetic technologies, such as *Escherichia coli,* to organisms that are not as amenable to genetic manipulation. Commonly plasmid RK2 is used to mobilise plasmids having a compatible origin of transfer *oriT*. This system is used for genetically modifying the important human pathogen *Clostridium difficile.* In this work we demonstrated *oriT* is not required for transfer between *E. coli* and *C. difficile* and that transfer is abolished in the presence of DNase indicating that a possible cell-to-cell transformation-like mechanism is responsible for transfer.

Objectives

Objectives

To investigate the mechanisms of transfer of plasmids between *E. coli* and *C. difficile*

Methods

Methods

E. coli donor strain CA434 (HB101 carrying the IncPß conjugative plasmid, R702) was transformed with pMTL9301 or pMTL9301Δ*oriT*. *E. coli* donors containing the plasmids was mixed with *C. difficile* CD37 and incubated anaerobically without selection for 18 hours then transferred onto selective plates, supplemented with the antibiotic to select for the plasmid-encoded resistance, with counter-selection against the *E. coli* donor.

Conclusions

Conclusions

Deletion of *oriT* lowered the transfer frequency of pMTL9301 but did not stop it showing that pMTL9301 can be transferred by a mechanism different from conjugation. In support of this pMTL9301Δ*oriT* could not transfer to *C. difficile* in the presence of DNase. We hypothesize that an unknown and undefined DNA uptake system, possibly a cell-to-cell transformation-like mechanism is involved.

FEMS7-2137
Physiology / Biochemistry / Molecular Microbiology - Part II

DEVELOPMENT OF A HOME BREW REAL-TIME PCR METHOD FOR RAPID DETECTION OF TOXIGENIC CLOSTRIDIUM DIFFICILE FROM CLINICAL SAMPLES

S. Khodaparast[1], A. Mohabati Mobarez[1], M. Mohammad Vasei[2], M. Mahmood Naderi[3], T. Tahereh Tohidi Moghadam[4], F. Savaheli Moghadam[1]
[1]Tarbiat Modares University, Medical Bacteriology, Tehran, Iran
[2]Shariati Hospital- School of Medicine- Tehran University of Medical Sciences, Department of Pathology, Tehran, Iran
[3]Digestive Disease Research Institute- Tehran University of Medical Sciences, Liver and Pancreatobiliary Diseases Research Center, Tehran, Iran
[4]Faculty of Biological Science- Tarbiat Modares University, Department of Nanobiotechnology, Tehran, Iran

Backgrounds

Qualification of *Clostridium difficile* DNA toxins (tcdA&tcdB) is used to monitor *C.difficile* infections. Although several commercial Real-time PCR methods are available for qualification of *C. difficile*, their major disadvantage is the sensitivity and accuracy of the assay.

Objectives

In the present study, an internally controlled qualificative Home brew multiplex Real-time PCR assay based on fluorescent labeled probes technology was developed for detection and qualification of *C. difficile* DNA toxins in stool samples.

Methods

To demonstrate its performance characteristics, a total of 186 stool samples were tested from patients hospitalized in gastroenterology, ICU and Bone marrow transplantation wards. Home brew multiplex Real-time PCR method and the commercial Real Star® altona.
Clostridium difficile PCR Kit assays were performed.TC using CCFA agar (MAST Group,England) was used a reference method.

Conclusions

The assay showed good accuracy and reproducibility. Total of 34 (18.3%) toxigenic *C.difficile* in stool samples were detected. Based on toxigenic culture, sensitivity and a specificity of Home brew method were 100% and 96.6% respectively.

Analysis of a panel containing potentially interfering bacteria demonstrated no cross reactivity with the assay. A strong correlation was observed between this Home brew multiplex Real-time PCR method and the commercial RealStar® altona Clostridium difficile PCR Kit (P = 0.000).
These results indicate that the affordable internally controlled Home brew multiplex Real-time PCR method described will be useful for monitoring and rapid detection of toxigenic *Clostridium difficile* from clinical samples of colitis patients.

FEMS7-0798
Physiology / Biochemistry / Molecular Microbiology - Part II

METAGENOMICS ANALYSIS OF THE GUT MICROBIAL POPULATION AND FUNCTIONALITY OF THE GRASSHOPPER, OXYA CHINENSIS SINUOSA

S.H. Hong[1], M. Shim[1], B.R. Kim[1], J. Shin[1], J. Lee[1], R.B. Guevarra[1], H.B. Kim[1]
[1]Dankook University, Animal Resources Science, Cheonan, Republic of Korea

Backgrounds

The gut microbiome of insect is known to play pivotal roles in its growth and development. In addition, the genetic diversity of the insect gut microbiota has recently been recognized as potential genetic resources for bioprocessing industry. However, limited information is available regarding the insect gut microbiome.

Objectives

The aim of this study was to get insight into the gut microbial population and functionality of the grasshopper, *Oxya chinensis sinuosa*.

Methods

Fecal samples from individual *O. c. sinuosa* (n = 10) were collected. The V5-V6 hypervariable regions of 16S rRNA genes and the whole metagenome were sequenced by using the Miseq chemistry. Sequences were quality assessed. A phylogenetic assessment was conducted using RDP classifier, and diversity indices were generated using QIIME with an Operational Taxonomic Unit definition at a similarity cutoff of 97%. Functional analysis of gut-derived microbiota was conducted by using the Microbial Genomics Module of CLC Genomics Workbench using the GO Term database Amigo2.

Conclusions

At the phylum level, the gut bacterial communities of *O. c. sinuosa* were dominated by *Firmicutes* and *Bacterioidetes* accounting for > 90% of total sequences. At the genus level, *Weissella, Acinetobacter, Arthrobacter, Lactococcus, and Pantoea* were dominant. The GO term analysis showed that functional properties related with xylan, cellulose, pectin catabolism of the gut-derived microbiota were abundantly present. The results from this study showed gut microbial populations and functionality of the *O. c. sinuosa*. These results establish a baseline toward the understanding of the insect gut microbiome, and provide us with fundamental knowledge for later studies.

FEMS7-1728
Physiology / Biochemistry / Molecular Microbiology - Part II

THE LAG PHASE OF E. COLI BL21(DE3) IN SUCCINATE MINIMAL MEDIUM SHORTENED BY MUTATIONAL ADAPTATION

H.J. Kim[1], H. Jeong[2], S.J. Lee[1]
[1]Chung-Ang University, Department of Systems Biotechnology, Anseong, Republic of Korea
[2]Korea Research Institute of Bioscience and Biotechnology, Infectious Disease Research Center, Daejeon, Republic of Korea

Backgrounds

As programmed in the genome, bacterial cells can adapt instantly and grow optimally under various environmental conditions. When cells encounter environmental stress, cellular growth will be delayed or lag phase will be prolonged. What happened in cells during the lag phase has been rarely studied, because enough cellular samples could not be obtained from the beginning stage of broth cultures. It has been just noted that the period of lag phase depends on the size and history of inoculum.

Objectives

The aim of this study was to confirm the microbial cellular systems can be reprogrammed by short-term adaptation via genomic mutations.

Methods

It was observed that the aerobic culture of BL21(DE3) cells in succinate minimal medium showed the extended lag phase compared to K-12 cells. We isolated large colonies in succinate minimal agar, which showed higher growth rates in succinate minimal broth. Next, genome sequencing characterized that such adaptations result from single genomic mutations in the regulatory region of a gene encoding a membrane protein. Seed dilution experiments showed those adaptive mutations occurred during the flask culture containing succinate minimal medium.

Conclusions

Accordingly, cellular system could be reprogrammed by adaptation via genomic mutations to change metabolic or regulatory network for optimal cellular growth when appropriate methods are not available in the genome information.

FEMS7-3038
Physiology / Biochemistry / Molecular Microbiology - Part II

WHOLE GENOME AMPLIFICATION AND SEQUENCING USING THERMOPHILIC DNA POLYMERASE

S. Kim[1]
[1]*korea Research Institute of Standards and Science, Center for bioanalysis, Daejeon, Republic of Korea*

Backgrounds

Whole genome amplification is a method of amplifying small amount of genomic material and used in a variety of applications such as single cell genomics, forensic science and etc. Currently, most of whole genome amplification is carried by the phi29 DNA polymerase which is derived from *Bacillus subtilis* bacteriophage. The phi29 DNA polymerase has exceptional processivity and strand displacement property and the genome can be amplified rapidly and isothermally by multiple displacement amplification. However, the phi 29 DNA polymerase has relatively high error rate and may introduce some errors during the whole genome amplification

Objectives

Investigation of whole genomic amplification using high-fidelity thermophilic DNA polymerase with bacterial genomic DNA and assessment of the amplified genomic materials.

Methods

The bacterial genomic DNA was extracted from *Escherichia coli* KCTC2441T and *Bacillus subtilis* 3135T using GenElute Bacterial Genomic DNA Kit (Sigma-aldrich). The amplification of the bacterial genome was carried out various condition and the amplified genome was sequenced using Illumina sequencer. The sequences reads were assembled using SPAdes and the assembly was assessed with QUAST.

Conclusions

The result suggested that whole genome amplification using high-fidelity thermophilic DNA polymerase can be used de novo single cell genomics or error-sensitive genomic analysis.

FEMS7-1690
Physiology / Biochemistry / Molecular Microbiology - Part II

DRUG DESIGN FOR HEPATITIS C VIRUS TREATMENT BASED ON SIRNA TECHNOLOGY

O. Koloskova[1], I. Shilovskiy[1], M. Khaitov[1]
[1]NRC Institute of Immunology FMBA Russia, Nanobiomedical Technologies, Moscow, Russia

Backgrounds

hepatitis C virus (HCV) affects about 3% of the world population. The disease is accompanied by damage to the liver and can lead to the death. Existing treatments have drawbacks, so new approaches for HCV treatment are an urgent task. A detailed study of the HCV life cycle molecular mechanisms has led to the possibility of design new gene therapeutics for this pathology treatment.

Objectives

design the novel complex based on siRNA for HCV treatment.

Methods

bioinformatics, PCR, in vitro gene silencing experiments.

Conclusions

there are three HCV genome regions (5'-UTR, NS2 and NS5B genes) which are the most conserved and involved in genome replication. So, these regions were chosen as a target sites for design of siRNA molecules. More than 100 different variants of siRNA molecules against these sites were predicted by OligoWalk program. 14 variants of designed siRNAs were synthetized and significantly downregulated the expression of these targets in vitro. The most potent siRNA targeted to 5'-UTR exhibited up to 90% suppression level.

For drug design the developed siRNA against 5'-UTR was encapsulated in cationic liposomes, modified by lactose derivative for the targeted delivery into the liver cells.

FEMS7-3105
Physiology / Biochemistry / Molecular Microbiology - Part II

PRELIMINARY FUNCTIONAL CHARACTERIZATION OF PUTATIVE TRANSCRIPTIONAL REGULATORS PA3027, PA3458 AND PA3973 FROM PSEUDOMONAS AERUGINOSA

K. Kotecka[1], *A.A. Bartosik*[1]
[1]*Institute of Biochemistry and Biophysics- Polish Academy of Sciences, Microbial Biochemistry, Warsaw, Poland*

Backgrounds

Pseudomonas aeruginosa is an opportunistic human pathogen, causing nosocomial infections. It is known for metabolic versatility, tolerance to a wide variety of physical conditions, antibiotic resistance mechanisms and complex regulatory systems. ParA and ParB proteins encoded by the majority of bacterial genomes are involved in chromosome segregation. In *P. aeruginosa* ParA and ParB are also directly or indirectly engaged in regulation of gene expression. PA3027, PA3458 and PA3973 are putative transcriptional regulators (TRs) from ParA/ParB regulon.

Objectives

The aim of this work was a functional characterization of PA3027, PA3458 and PA3973. One of the tasks was construction of chromosomal mutants and their phenotypic analysis. Another task concerned analysis of proteins and their regulatory properties *in vivo* and *in vitro*.

Methods

We applied: site-directed mutagenesis, phenotypic characterization (growth, morphology, motility, phenotype microarrays, biofilm formation), protein overproduction and *in vitro*/*in vivo* regulatory experiments.

Conclusions

The chromosomal single deletion mutants in TR genes and double mutants in one of TR gene and *parB* are not lethal for *P. aeruginosa.* The TR mutants do not show significant differences in tested phenotypes when compared to the wild type *P. aeruginosa*. The purified HIS-tagged proteins form dimers *in vitro*. The *PA3458* has the strongest promoter out of three included in the analysis, active in *P. aeruginosa* and *E. coli*. Overproduction of PA3458, but not PA3027 or PA3973 has the negative impact on the growth of *P. aeruginosa*, but not *E. coli.*

This work was supported by the National Science Centre in Poland (grant 2015/18/E/NZ2/00675).

FEMS7-0836
Physiology / Biochemistry / Molecular Microbiology - Part II

ADAPTATION OF SINORHIZOBIUM MELILOTI TO NCR PEPTIDES COUPLED WITH INCREASED RESISTANCE TO ANTIMICROBIAL AGENTS

E. Kovács[1], *É. Kondorosi*[1], *A. Kereszt*[1]
[1]*Biological Research Center, Biochemistry Institute, Szeged, Hungary*

Backgrounds

Since the occurrence of antibiotic-resistant superbugs resulting from the widespread use of conventional antibiotics is multiplying, numerous studies have focused on the use of antimicrobial peptides (AMPs) as therapeutic agents.

A large group of AMP-like molecules with up to 600 different members is the nodule-specific cysteine-rich (NCR) peptide family, which is produced almost exclusively in the infected cells of the nitrogen-fixing nodules of *Medicago truncatula*.

Objectives

Cationic NCR peptides have been found to have antimicrobial activity, killing *Sinorhizobium meliloti* (and other Gram-negative and Gram-positive bacteria as well as fungi) when applied at high concentration.

Methods

We evolved increased tolerance against a peptide in the wild-type and the *bacA* mutant strains of *S. meliloti* 1021 by increasing gradually the concentration of NCR335 in the growth medium. The lines became tolerant not only towards the NCR335 peptide but also against other cationic peptides such as NCR247 and indolicidin isolated from bovine neutrophils. Genome sequencing of the peptide-tolerant lines revealed that they accumulated 5-10 mutations during their evolution. To test the effect of the individuals mutations on peptide sensitivity both in free-living state and *in planta*, they have been transferred into the genome of parental strains as well as the mutant genes cloned in a plasmid have been expressed from the *bacA* promoter.

Conclusions

The mutations can render the cells to tolerate 2-3 times higher concentration of NCR peptides and other antimicrobial drugs than the wild type strain.

FEMS7-1057
Physiology / Biochemistry / Molecular Microbiology - Part II

NEW IMPORTANT GENES IN CHRONIC INTRACELLULAR INFECTION OF SINORHIZOBIUM MELILOTI

E. Kovács[1], *É. Kondorosi*[1], *A. Kereszt*[1]
[1]*Biological Research Center, Biochemistry Institute, Szeged, Hungary*

Backgrounds

Sinorhizobium meliloti differentiates nitrogen-fixing bacteroids in root nodules of the legume *Medicago truncatula*. Nodule-specific cysteine-rich antimicrobial peptides (NCR AMPs) and the bacterial BacA protein are crucial for bacteroid development. Cationic NCR peptides have been found to have antimicrobial activity in vitro, killing *S. meliloti* when applied them at high concentration. To counteract this antimicrobial activity, rhizobia requires the BacA protein. In the absence of this protein, the bacteroids do not differentiate, rather they are immediately killed by the NCR peptides in the nodule as soon as they are released in the symbiosomes. In vitro *bacA* deficient cells were hypersensitive to NCRs.

Objectives

It seemed to be relevant question that NCR-tolerance affected bacteroid development.

Methods

We evolved increased tolerance against NCR335 in *bacA* mutant strain using 10 parallel lines. These mutant lines became tolerant to NCR335, polimyxin B, indolicidin and NCR247. After genome sequencing of the peptide-tolerant lines, the effects of six different mutations were tested in vitro and in planta. A few among these mutations could help *S. meliloti bacA* deficient cells to survive in the milieu filled with antimicrobial peptides which means that the bacteroid differentiation and the formation of nitrogen-fixing nodules were complete.

Conclusions

The mutations can render the cells to tolerate high antimicrobial drug concentration and help rescue *S. meliloti bacA* deficient cells.

FEMS7-0745
Physiology / Biochemistry / Molecular Microbiology - Part II

INVESTIGATION OF THE ROLE OF BFMR REGULATOR IN CLINICALLY IMPORTANT FEATURES OF OPPORTUNISTIC PATHOGEN ACINETOBACTER BAUMANNII

R. Krasauskas[1], J. Skerniškytė[1], J. Armalytė[1], E. Sužiedėlienė[1]
[1]Life Sciences Center- Vilnius University, Institute of Biosciences, Vilnius, Lithuania

Backgrounds

In recent years, *Acinetobacter baumannii* emerged as an important Gram-negative opportunistic pathogen that plays a great role in hospital-acquired infections. Previously, it has been shown that one of the clinically relevant traits – biofilm formation – of *A. baumannii* is controlled by the response regulator BfmR which, along with histidine kinase BfmS, comprises the two-component signal transduction system BfmRS. However, data on the precise role of BfmR in the regulation of biofilms and other clinically important features is in scarce.

Objectives

To assess the role of BfmR in *A. baumannii* physiology using various mutant alleles.

Methods

Whole *bfmRS* operon was deleted in a clinical strain of *A. baumannii* after which the strain was complemented with various mutant alleles of *bfmR* regulator placed under an inducible promoter. Clinically important phenotypes such as motility, biofilm and pellicle formation were assessed using various inducer concentrations.

Conclusions

We determined, that wild type *bfmR* allele (WT) displays concentration independent biofilm and pellicle induction. However, surface associated motility of *A. baumannii* was variable and was completely abolished under high *bfmR* induction. We also obtained a mutant of *bfmR* which behaves more like an attenuated WT allele – biofilms, pellicles, and motility are complemented only at a particular inducer concentration. The results suggest that the role of BfmR in *A. baumannii* physiology might be concentration-dependent and that we may have obtained at least a fraction of its activity spectrum. They also provide us with the tools to investigate the BfmRS regulon under different biofilm inducing conditions.

FEMS7-2890
Physiology / Biochemistry / Molecular Microbiology - Part II

CATALYTIC PROPERTIES OF S1-LIKE AND DNASE II-LIKE NUCLEASES FROM ACANTHAMOEBA CASTELLANII.

R. Krela[1], M. Węglewska[2], E. Poręba[2], K. Leśniewicz[1]
[1]Adam Mickiewicz University in Poznan, Department of Molecular and Cellular Biology, Poznan, Poland
[2]Adam Mickiewicz University in Poznan, Department of Molecular Virology, Poznan, Poland

Backgrounds

Free-living amoeba *Acanthamoeba castellanii* is an opportunistic animal pathogen, causal agent of amoebic keratitis and granulomatous amoebic encephalitis (GAE). It is ubiquitous in environment and play crucial role in nitrogen and carbon flow in soil, where it grazes on bacteria. The life cycle of *A.castellanii* is divided into vegetative trophozoite stage and dormant cystic stage. Due to such diverse mode of action and food source, it is important to examine enzymes involved in feeding of this protozoa. Recently there was a many reports on different proteins crucial for phagocytosis, encystation and pathogenicity. Although any nuclease has not been examined, despite fact that high nucleolytic activity of *Acanthamoeba castellanii* was shown as a characteristic trait of this organism.

Objectives

Analysis of *Acanthamoeba castellanii* nuclease activity during life cycle and characterization of involved enzymes.

Methods

Using nuclease activity in-gel assay we examined cell lysate and medium of different time points of *Acanthamoeba castellanii* culture. Based on genome sequence we determined genes and possible proteins responsible for high nucleolytic activity of *A.castellanii*. We overexpressed DNAse II-like and S1/P1-like nucleases using mammalian cells system. Analyzed activity and catalytic properties of this distinct nucleases by zymography.

Conclusions

Acanthamoeba castellanii has two distinct groups of DNA-degrading nucleases. S1/P1-like enzymes with homology to fungi and plants nucleases involved in PCD, and DNAse II-like nucleases with homology to animals enzymes. It is first reported organism with nucleases from both families. Although their functions in *Acanthamoeba castellanii* still remain unclear.

This research is supported by the Dean of the Adam Mickiewicz University Faculty of Biology, grant GDWB 10/2016.

FEMS7-0046
Physiology / Biochemistry / Molecular Microbiology - Part II

COPPER NANOPARTICLES EXERT THEIR ANTIMICROBIAL PROPERTIES BY PREVENTING Z-RING FROMATION

G. Kumar[1], S.D. Pandey[1], S. Mallick[1], S. Ghosh[1], P. Pramanik[1], A. Ghosh[1]
[1]Indian Institute of Technology Kharagpur, Biotechnology, Kharagpur, India

Backgrounds

In recent years, copper nanoparticles (CuNPs) have been used as alternative to challenge the menace of MDR bacteria to prevent nosocomial infections. Though the cost effectiveness and low dosage requirement CuNPs has gained importance in antimicrobial applications, insight in its mode of action remains unknown.

Objectives

1. Assessment of antimicrobial activity of newly synthesized CuNPs
2. Understanding the bactericidal mechanism of CuNPs.

Methods

The stable CuNPs of size 5-45 nm were synthesized by chemical reduction method and characterized physio-chemically using XRD and TEM. The antimicrobial and anti-biofilm properties of CuNPs were assessed (MIC and MBEC) where it showed the ability to sensitize bacterial cells and reduced biofilm formation. Localization studies with ectopically expressed GFP-tagged FtsZ and FtsI in *E. coli* in presence of CuNPs revealed the appearance of elongated cells without Z-rings or medially localized FtsI, indicating the possible involvement of CuNPs in preventing the Z-ring formation. The CuNPs were also able to degrade DNA from both mammalian and bacterial origin possibly due to generation of reactive oxygen species (ROS) as the expression of oxidative stress defence genes,e.g., *ahpC* of *Mycobacterium smegmatis* was significantly high in CuNPs treated cells as revealed by synthesized cDNA by semi quantitative RT-PCR and observing its degradation. However, cytotoxicity (Methyl Tri Chloro Trifluoro Tetrazolium Bromide or MTT) assay of CuNPs showed toxicity towards the RAW 264.7 cells.

Conclusions

The CuNPs synthesized have significant anti-microbial and anti-biofilm activities. Here, we hypothesize that CuNPs might prevent Z-ring formation to inhibit bacterial division and causesDNA degradation possibly through ROS generation.

FEMS7-0705
Physiology / Biochemistry / Molecular Microbiology - Part II

IMPACT OF POINT MUTATION S191A OF NDM-7 OF KLEBSIELLA PNEUMONIAE ON ITS ANTIMICROBIAL SUSCEPTIBILITY AND PROTEIN STABILITY

G. Kumar[1], I. BAGRE[2], D. Kar[1], S. Biswal[1], A.S. Ghosh[1]
[1]Indian Institute of Technology Kharagpur, Biotechnology, Kharagpur, India
[2]Université Félix Houphouët-Boigny, UFR Biosciences, Abidjan, Ivory Coast

Backgrounds

New Delhi Metallo beta-lactamase (NDM) is of significant public health concern due to its enormous potential to hydrolyse all major beta-lactams including carbapenems. Amino acid substitutions outside the active site reportedly affect NDM beta-lactamase activities.

Objectives

In this work, we attempt to identify specific mutations that can influence the beta-lactam hydrolysing ability of NDM-7.

Methods

bla$_{NDM-7}$ was cloned from clinical isolates in pET and pBAD vectors, and mutated *bla*$_{NDM-7}$ constructs were created by site-directed mutagenesis. Recombinant NDM-7 and its mutant were purified via Ni-NTA metal based affinity chromatography. The differences in proteins *in vitro* biochemical and biophysical properties were analysed via steady state kinetics studies with various beta-lactams and analysed through mass spectroscopy and circular dichroism spectra analysis. The cellular effects of the mutated proteins were investigated by determining the beta-lactam sensitivity alterations in presence and absence of ethylenediaminetetraacetic acid (EDTA) as EDTA chelates zinc ions that are required for NDM-7 activity.

Conclusions

Among the different substitution mutations, serine at position 191 is identified as the most influencing residue for NDM-7. The beta-lactam resistance of NDM-7 is remarkably affected upon S191A substitution and the results are in synchrony with the modulations in kinetic parameters. Normally, NDM-7 hydrolyzes beta-lactams efficiently, but NDM-7_S191A has lost its ability to hydrolyze the beta-lactams tested, especially penicillins and carbapenems. Though, the substitution did not affect the overall folding pattern of the NDM-7, substantial differences in thermal stabilities are observed. Therefore, we hypothesize that Ser191 plays a crucial role in exhibiting beta-lactamase nature of NDM-7.

FEMS7-0321
Physiology / Biochemistry / Molecular Microbiology - Part II

OUTER MEMBRANE VESICLE PRODUCTION IN BACTEROIDES FRAGILIS IS REGULATED BY DNA INVERSION

T. Kuwahara[1], *H. Nakayama-imaohji*[1]
[1]*Kagawa University, Microbiology- Faculty of Medicine, Kagawa, Japan*

Backgrounds

Phase changes in gut microbe *Bacteroides fragilis* mediate variations in a vast array of cell surface molecules. Three master DNA invertases that globally control promoter inversions at many distant regions have been identified in *B. fragilis*: Mpi, Tsr0667 and BF2766. BF2766 inverts two distantly localized promoters, IVp-I and IVp-II.

Objectives

BF2766-regulated invertible promoters of *B. fragilis* were further analyzed to determine the role of their associated proteins in surface structure modification.

Methods

A series of BF2766 mutants were constructed in which the two promoters were locked in different configurations (IVp-I/IVp-II=ON/ON, OFF/OFF, ON/OFF or OFF/ON). DNA microarray analysis was performed with these mutants to identify genetic regions whose alterations in expression were specifically associated with ON/ON genotype. We compared the survival of the four mutants following contact with antimicrobial compounds (bile, LL-37 and human defensins).

Conclusions

ON/ON *B. fragilis* mutants exhibited hypervesiculating, whereas the other mutants formed only a trace amount of outer membrane vesicles (OMVs). By comparing the transcriptomes of the four BF2766 deletion mutants, we found that the transcription of the genes downstream of IVp-II markedly elevated in a hypervesiculating ON/ON strain. The ON/ON mutants showed higher resistance to treatment with bile, LL-37, and human b-defensin 2. Incubation of wild-type cells with 5% bile increased the population of cells with the ON/ON genotype. These results indicate that *B. fragilis* regulates the formation of OMVs through DNA inversions in response to membrane stress.

FEMS7-1530
Physiology / Biochemistry / Molecular Microbiology - Part II

EXPRESSION OF GROEL PROTEIN OF BURKHOLDERIA MALLEI USING A SYNTHETIC DNA FRAGMENT

S. Kye[1], J. You[1], J. Ha[1]
[1]Animal and Plant Quarantine Agency, Infectious Diseases Department, Seoul, Republic of Korea

Backgrounds

Burkholderia mallei is a gram-negative bacterium that causes glanders, a disease primarily affecting Equidae, which is also a fatal zoonotic infectious disease. Current veterinary diagnosis relies on the crude preparation of the B. mallei antigen. This reagent not only poses a category B biothreat during its preparation, but also causes a considerable number of false results. The preparation of an alternative reliable antigen will improve the diagnosis of glanders.

Objectives

We attempted to produce the GroEL protein of B. mallei, which is easily accessible in diagnostic laboratories and of diagnostic value.

Methods

We synthesized in vitro DNA fragment of the B. mallei GroEL gene based on the sequences previously published. The synthetic DNA fragment was expressed in a baculovirus expression system. Antigenic property of the expressed protein was investigated using the positive sera.

Conclusions

The recombinant GroEL protein produced in this system shared the similar antigenicity to the existing crude antigen of B. mallei, displaying its diagnostic value for the detection of B. mallei. However, further validation using a large number of field sera is needed to be employed in the diagnostic program.

FEMS7-0592
Physiology / Biochemistry / Molecular Microbiology - Part II

TAXOGENOMIC STUDY OF MARINE BACTERIA BELONGING TO THE FAMILY RHODOBACTERACEAE

A. La Mura[1], *M.J. Pujalte*[1], *D. Ruiz*[1]
[1]*Universitat de Valencia, Departamento de Microbiología y Ecología, Valencia, Spain*

Backgrounds

Comparative genomics holds great potential for prokaryote systematics since it allows to perform phylogenetic analyses based on the whole genome content. Furthermore it might assist to improve the general biological knowledge of the organisms through the reconstruction of metabolic pathways. However, and spite considerable progress in recent years many prokaryotic lineages still lack sequenced genomes, which is desirable, at least for the type strain of each species. And for the genomes that are available there is a bias toward organism of medical importance.

Objectives

In this project we want to obtain draft genomes of 50 type strains from the Spanish Type Cultures Collection CECT, all of them related to the marine environment and belonging to different genera of the family *Rhodobacteraceae*. A second aim is to make comparative analysis together with genomic sequences and assemblies from public repositories. As part of these analyses we want to explore phenotypic inferences.

Methods

The methods include laboratory and bioinformatic work:

1. Laboratory techniques: cultivation of strains, extraction and purification of DNA.
2. Bioinformatics work: raw data processing, quality control, de novo assembly, calculation of similarity indexes, functional annotation and search of metabolic pathways.

Conclusions

The present work is intended for the Doctoral Thesis of the presenting author. At present only the selection of strains and a first batch of next generation sequencing has been undertaken. We expect to communicate more results during the event.

FEMS7-3144
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERIZATION OF THE CONTAMINANT BACTERIAL COMMUNITIES IN SUGARCANE FIRST GENERATION (1G) INDUSTRIAL ETHANOL PRODUCTION

C. Labate[1], M.L. Bonatelli[1], M.C. Quecine-[1], M.S. Silva[1]
[1]University Of Sao Paulo / Luiz de Queiroz College of Agriculture, Departamento de Genética, Piracicaba, Brazil

Backgrounds

The industrial ethanolic fermentation process is operated in distilleries, either in fed-batch or continuous mode. A consequence of the large industrial production of ethanol is bacterial contamination in the fermentation tanks, which is responsible for significant economic losses. Thus, we accessed the profile of bacterial communities from two distilleries in Brazil; one operating in fed-batch and another in continuous mode to ethanol production. Both ethanol production mode showed similar bacterial density, around 10^5 gene copies/mL.

Objectives

This work focused on the cultured-independent assessment and characterization of contaminant bacterial community from sugarcane ethanol fermentation process. To this purpose, two distinct distilleries in the state of São Paulo were sampled during the harvest season of 2012-2013. To our knowledge, this is the first cultured-independent assessment of this microbiota.

Methods

Both ethanol production mode showed similar bacterial density, around 10^5 gene copies/mL. However, 16S rDNA sequencing showed differences in the bacterial profile between the two distilleries. It was reported 219 genera that belong to 12 different phyla, several of them never described in this environment. As expected, 91% to 99% of the sequences were affiliated to the *Lactobacillus* genus. Alpha diversity only showed a correlation through the fermentation tanks in continuous mode. Beta diversity clearly separated the two distilleries and the operational taxonomy units (OTU) that were differentially represented belonged mainly to *Lactobacillus*, whereas several of them were unique to the continuous mode.

Conclusions

The *Lactobacillus* genus may be even more important to this environment than ever thought, that each distillery appears to have a distinct microbiome, considering both OTU and predictive gene families, and that this community seems to persist a long time. These results suggest that the study of the contaminant microbiota, from a particular distillery, may be the way to find customized and less expensive solutions to control them.

FEMS7-3177
Physiology / Biochemistry / Molecular Microbiology - Part II

ISCR1 CONTRIBUTES TO ANTIBIOTIC RESISTANCE

C. Lallement[1], *T. Jové*[1], *M.C. Ploy*[1]
[1]*UMR Inserm S-1092, Université de Limoges, Limoges, France*

Backgrounds

The IS*CR* family of bacterial insertion sequences (IS) consists in a group of poorly characterized IS related to IS*91*. They encode an HUH transposase though to catalyze the rolling circle transposition of these families. It is admitted that these elements contribute to dissemination and contribution to the expression of the antibiotic resistance. The prevailing IS*CR*, IS*CR1,* shows a large repertoire of combinations of genes downstream and particularly, antibiotic resistance genes. Moreover, this element provides two outward-oriented promoters suggested in literature, located in a region at the 3' of the element (*ori*IS). The role of IS*CR1* in antibiotic resistance contribution is yet to be clarified.

Objectives

The aims of the study is to investigate (I) if IS*CR1* element contributes to the antibiotic resistance and (ii) to characterize of promoter(s) involved.

Methods

Three antibiotic resistance genes (*qnrA1*, $bla_{CTX\text{-}M\text{-}9}$ and *dfrA19*) located at different distance from IS*CR1* 3' end, were studied. For each of them, *lacZ* transcriptional fusions of their intergenic region with or without *ori*IS region (IS*CR1*) were performed. Promoters activity were measured by β-galactosidase assays. Besides, in parallel, phenotypic assays were performed with each genes and +/- *ori*IS region.

Conclusions

*ori*IS region increases to the expression of those three genes (4-15 fold). When *lacZ* gene was replaced by the *blaCTX-M-9* gene, the presence of *ori*IS presence was essential to provide a resistance phenotype. Therefore, IS*CR1* is involved in process of antibiotic resistance which may explain the epidemiological success of IS*CR1* in bacteria.

FEMS7-1332
Physiology / Biochemistry / Molecular Microbiology - Part II

BIOCHEMICAL AND FUNTIONAL ANALYSIS OF THE RECOMBINANT ZUR MAP3773C OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

B. Landeros Sanchez[1], G. Ibarra Molina[1], J.A. Gutierrez-Pabello[2], L.A. Hurtado Ayala[1], M.D.C. Brito Perea[1], A. Fernandez Otal[3], M. Fillat[4]
[1]Universidad Autonoma de Baja California, Facultad de Ciencias Quimicas e Ingenieria, Tijuana- B.C., Mexico
[2]Universidad Nacional Autónoma de Mexico, Facultad de Medicina Veterinaria y Zootecnia, Mexico, Mexico
[3]Instituto Aragonés de Ciencias de la Salud,
Department of Biochemistry and Molecular and Cell Biology. Faculty of Sciences and Institute for Bioc omputation and Physics of Complex Systems, Zaragoza- Spain, Spain
[4]University of Zaragoza,
Department of Biochemistry and Molecular and Cell Biology. Faculty of Sciences and Institute for Bioc omputation and Physics of Complex Systems, Zaragoza- Spain, Spain

Backgrounds

Paratuberculosis (PTB) is caused by *Mycobacterium avium subsp. paratuberculosis* (MAP). The study of protein functionality and characterization is an important issue to elucidate its possible use in vaccine design.

Objectives

This study was designed to characterize the functionality and biochemical properties of the Zur MAP3773c protein

Methods

We performed the purification of the recombinant protein using a zinc-loaded IMAC column, and determined its structural content of zinc, using PAR staining. Oligomeriztion properties were analysed by, crosslinking with gluraldehyde and its self-regulation was assesed by EMSA. Partial structure of Zur MAP3773c was investigated by circular dichroism using DicroWeb.
Results. The recombinant MAP3773c protein presented the following biochemical characteristics: Absence of prosthetic group, non-formation of intramolecular bond oligomers according to DTT analysis, no formation of intermolecular bond oligomers after glutaraldehyde crosslinking, absence of structural zinc and Zn^{2+} dimerization. For secondary structure, the predominance alpha helix constituted around 50%, versus the 20% of the folded beta sheet. Around 23% corresponded to structurally disordered regions, in good concordance with the characteristics of crystal structures available from other FUR proteins. Furthermore, MAP3773c is a self-regulated protein.

Conclusions

MAP3773c is a Zur protein that is able to bind to *E. coli* iron boxes. Interaction of MAP3773c with the mycobacterial box found in its own promoter indicates that the MAP3773c protein is self-regulated.

FEMS7-2757
Physiology / Biochemistry / Molecular Microbiology - Part II

SYNTHESIS OF AMONABACTINS BY AEROMONAS CATALYZED BY A NONRIBOSOMAL PEPTIDE SYNTHETASE WITH UNIQUE ITERATIVE-ALTERNATIVE-OPTIONAL MECHANISM

Q. Esmaeel[1], M. Chevalier[1], P. Jacques[1], V. leclere[1]
[1]Univ Lille - Sciences et technologies, Institut Charles Viollette, villeneuve d'ascq, France

Backgrounds

Aeromonas are Gram negative bacteria, fish pathogens, commonly found in aquatic environments. Pathogenicity and virulence of *A*. *hydrophila* and *A*. *salmonicida* strains are related to the ability to produce virulence factors as siderophores. Amonabactins represente a family of 4 peptide-based siderophores co-produced by *Aeromonas*. They are composed of 5 or 6 monomers including 2 DhB, 2 Lys, an aromatic residue (either D-Trp or D-Phe), and an optional Gly. As many bacterial siderophores, they are synthesized by a non-ribosomal peptide synthetase (NRPS).

Objectives

The objective was to understand how a single NRPS can lead to the co-production of 4 amonabactins

Methods

The domain architecture of proteins constituting the nonribosomal assembly line was determined using specific bioinformatics tools. Hypothesized mechanism of biosynthesis was deciphered by analyzing the production of the 4 different amonabactin forms from mutants deleted in different domains.

Conclusions

From *A. hydrophila* genome sequence, the amonabactin synthesis operon was identified. It is constituted of 7 genes we have named *amo*CEBFAGH, including 4 NRPS genes. The same *amo* cluster was found widespread among most of the *Aeromonas* species. Knockout mutants in *amo*G and *amo*H genes were constructed allowing the deciphering of a unique mode of synthesis, the most complex described so far for NRPS. It was qualified of i) alternative as Phe or Trp can be equally recruited by the same A-domain of AmoG, ii) iterative as AmoE and AmoF work twice to assemble two [Dhb-Lys], iii) optional as Gly incorporated by AmoH is only present in 2 over the 4 amonabactins.

FEMS7-1499
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERIZATION OF A THERMOSTABLE A-GALACTOSIDASE INVOLVED IN RAFFINOSE FAMILY OLIGOSACCHARIDE UTILIZATION FROM CALDICELLULOSIRUPTOR BESCII

A. Lee[1], S. Suzy[1], L. Junho[1], C. Jaeho[1]
[1]Pusan National University, Microbiology, Busan, Republic of Korea

Backgrounds

Caldicellulosiruptor bescii, which belongs to Gram-positive and thermophilic bacteria, was isolated from hot spring in Russia. *C. bescii* has a high capacity to utilize various carbohydrates and harbors 88 genes related to carbohydrate utilization. Among them, the gene cluster proposed to be involved in the degradation of the raffinose family oligosaccharides (RFO) were detected. The RFO, which are α-(1,6) galactosyl derivatives of sucrose, play role in storage or transportation of carbohydrates in mature seeds and leaves of the plants. The bacteria capable of utilization of RFO has rarely studied in environmental sources, but *C. bescii* showed growth on raffinose as carbon source.

Objectives

To understand how *C. bescii* can utilize the RFO, we decided to investigate the proteins related to raffinose transport and hydrolysis.

Methods

As the first step, the gene encoding a putative α-galactosidase (CbAga36) was cloned and expressed in *Escherichia coli*. Size exclusion chromatography of the purified rCbAga36 indicated that the native form was a tetramer. The purified rCbAga36 was optimally active at pH 5.0 and 70°C and had a half-life of 15h at 70°C. rCbAga36 exhibited high activity with artificial substrate compared to the natural substrates such as melibiose and raffinose. rCbAga36 exhibited preferential activity toward the raffinose and stachyose, but did not degrade the galactomannans.

Conclusions

Our results imply that CbAga36 may play a role for degradation of RFO into galactose, further utilized as an energy source in *C. bescii*. Furthermore, its ability for synthesizing novel oligosaccharides by transglycosylation reaction makes it potentially useful for functional food.

FEMS7-0911
Physiology / Biochemistry / Molecular Microbiology - Part II

SCOLOPENDIN LEADS CA2+-MEDIATED MITOCHONDRIAL DYSFUNCTION AND CASPASE DEPENDENT APOPTOTIC DEATH IN CANDIDA ALBICANS

H. Lee[1], *D.G. Lee*[1]
[1]*Kyungpook National University, School of Life Sciences, Daegu, Republic of Korea*

Backgrounds

Centipedes, a kind of arthropod, reportedly produce antimicrobial peptides as part of an innate immune response. Scolopendin (SRSEKAGLQFPVGRIGRMLKK) is a novel antimicrobial peptide derived from the body of *Scolopendra subspinipes mutilans* centipede by using RNA sequencing.

Objectives

Many antifungal agents have more than one type of cell death mechanism. Although scolopendin is involved in membrane perturbation, the corresponding intracellular changes require further investigation. To address the critical need for novel antimicrobial therapeutics, we have rationally investigated induction of yeast apoptotic death of scolopendin.

Methods

We assessed the cell morphology and calcium ion concentration of the cytosol and mitochondria of scolopendin-treated cells. To investigate the intracellular responses induced by scolopendin, ROS accumulation were monitored. Cells exposed to scolopendin were identified using diagnostic markers of apoptotic response including phosphatidylserine externalization, DNA fragmentation, and nuclear condensation.

Conclusions

Treatment of *Candida albicans* with scolopendin induced the apoptotic response, which in turn led to mitochondrial dysfunction, metacaspase activation, and cell death.The elucidated mechanisms responsible for the antifungal activity of scolopendin provide possible applications in fighting clinical fungal infections.

FEMS7-0368
Physiology / Biochemistry / Molecular Microbiology - Part II

GLUCOSE-DEPENDENT REGULATION OF THE STRINGENT RESPONSE IN ESCHERICHIA COLI

J.W. Lee[1], Y.H. Park[2], Y.J. Seok[3]
[1]Seoul National University, Biophysics and Chemical Biology, SEOUL, Republic of Korea
[2]Seoul National University, Biological Sciences and Institute of Microbiology, SEOUL, Republic of Korea
[3]Seoul National University,
Biophysics and Chemical Biology- Biological Sciences and Institute of Microbiology, SEOUL, Republic of Korea

Backgrounds

Bacteria respond to various stresses by modulating the level of alarmone (p)ppGpp (guanosine tetraphosphate and pentaphosphate) through a process called the stringent response. In *Escherichia coli*, the level of (p)ppGpp is regulated by stringent factors, RelA and SpoT proteins. RelA catalyzes the synthesis of (p)ppGpp, whereas SpoT catalyzes both the synthesis and hydrolysis of (p)ppGpp, serving as a bifunctional enzyme.

Objectives

SpoT is particularly important in balancing the intracellular level of (p)ppGpp since it is the only enzyme responsible for (p)ppGpp hydrolysis. However, the underlying mechanisms for the (p)ppGpp hydrolase activity regulation of SpoT still remain unknown in *E. coli*. In this study, we conducted ligand-fishing experiment and found Rsd as a novel interaction partner of SpoT.

Methods

We identified specific interaction between SpoT and Rsd using ligand-fishing experiment and BACTH (Bacterial Two Hybrid) assay.

Measuring intracellular (p)ppGpp concentration using TLC analysis in Rsd overexpressing strain.

Growth test under starvation condition.

Conclusions

HPr is a general PTS component and participates in the phosphorylation-coupled transport of numerous sugars. We recently reported that HPr is dephosphorylated and interacts with Rsd when a favorable carbon source, such as glucose, is available. In this study, we demonstrate that only dephosphorylated form of HPr, but not phosphorylated HPr, can sequester Rsd from SpoT to antagonize its stimulatory effect on SpoT. Based on these data, we suggest that SpoT-mediated stringent response can be regulated by Rsd in a glucose-dependent manner, proposing a novel stringent response mechanism in *E. coli*.

FEMS7-0793
Physiology / Biochemistry / Molecular Microbiology - Part II

FRAS (FERMENTATION-RESPIRATION SWITCH) EXPRESSION IS INDUCED UNDER ANOXIC CONDITION VIA OXYGEN-FNR-SRNA REGULATORY PATHWAY

K.H. Lee[1], B.R. Jang[1], K.J. Lee[1], S.J. Park[2]
[1]Sogang University, Department of Life Science, Seoul, Republic of Korea
[2]Yonsei University College of Medicine, Department of Environmental Medical Biology, Seoul, Republic of Korea

Backgrounds

An enzyme, FrsA converting pyruvate to acetaldehyde and carbon dioxide has been identified in *Vibrio vulnificus*. Since FrsA protein was rarely detectable in cells grown under oxygen-rich condition, the *in vivo* activity of FrsA was observed in cells grown under anaerobic condition.

Objectives

To investigate the regulatory mechanism for anaerobic induction of FrsA, *frsA* transcription was monitored, but no significant difference in its transcription was observed in the cells grown under aerobic or anaerobic conditions, suggesting the involvement of posttranscriptional regulation in FrsA expression.

Methods

In silico analysis of *V. vulnificus* genome showed a tentative sRNA whose sequence is complementary to 5'-UTR of *frsA* mRNA. A northern blot revealed the presence of 350 nucleotide-long sRNA. Regulatory role of this sRNA was examined using the mutant deficient in the sRNA. The mutant showed high level of FrsA protein and activity of pyruvate decarboxylase even under the aerobic condition. Thus, it was named as the regulatory sRNA for FrsA expression (Rsf). Regulatory dependency of Rsf on oxygen was further examined in repressing FrsA expression. *rsf* gene expression was repressed by a transcription factor FNR under anaerobic condition, whereas repression of *rsf* transcription by FNR was relieved in the presence of oxygen.

Conclusions

Therefore, this study demonstrates that the cellular content of FrsA is minimized during aerobic growth period via repression of its expression by Rsf. Under anaerobic condition, however, FrsA expression is derepressed due to repression of *rsf* transcription by FNR, resulting in increased fermentative metabolism(s) of *V. vulnificus*.

FEMS7-2761
Physiology / Biochemistry / Molecular Microbiology - Part II

ACETATE FLUXES IN ESCHERICHIA COLI ARE DETERMINED BY THE THERMODYNAMIC CONTROL OF THE PTA-ACKA PATHWAY

B. Enjalbert[1], P. Millard[1], M. Dinclaux[1], J.C. Portais[1], F. Letisse[1]
[1]Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, Metasys, Toulouse, France

Backgrounds

It is known that *Escherichia coli* excretes acetate when growing on excess fermentable sugars. It is consumed once the glucose is totally depleted in the medium. This diauxic behavior is due to the catabolite repression exerted by glucose on acetate utilization. Acetate consumption is prevented by the repression of acetyl-coA synthetase (*acs*) expression, considered as the main pathway for acetate metabolization. However, it was recently observed that acetate can be taken up and metabolized during exponential growth of *E. coli* K-12 strains on a mixture of glucose and acetate[1]. The ability of *E. coli* to consume acetate in such conditions is highly intriguing since *acs* is not expressed due to catabolite repression. This observation suggests that acetate can still be utilized by another pathway upon glucose excess.

Objectives

We first aimed to clarify the pathway by which acetate is consumed upon growth on excess glucose and then to identify the mechanism(s) that control(s) acetate metabolism in such conditions.

Methods

To address these questions, we designed and carried out dynamic ^{13}C-labeling experiments to quantify acetate production and consumption fluxes individually, and to identify the metabolic pathways supporting the fluxes.

Conclusions

The results pointed out that the constitutive Pta-AckA pathway (*pta*, phosphate acetyl-transferase; *AckA*, acetate kinase) was responsible for both fluxes, and thermodynamic and *in silico* kinetic analyses suggested this pathway is thermodynamically controlled *in vivo* by extracellular acetate level. Catabolite repression is hence not the unique determinant of acetate utilization. The regulatory mechanism proposed was validated experimentally on glucose and two other glycolytic substrates.

FEMS7-2502
Physiology / Biochemistry / Molecular Microbiology - Part II

DICEPHERING THE ROLE OF MULTIPLE THIOREDOXINS FOLD PROTEINS OF LEPTOSPIRILLUM SP. CF-1 IN OXIDATIVE STRESS TOLERANCE

G. Levicán[1], D. González[1], P. Álamos[1], O. Orellana[2], R. Chávez[1]
[1]Universidad de Santiago de Chile, Biología, Santiago, Chile
[2]Universidad de Chile, Instituto de Ciencias Biomédicas, Santiago, Chile

Backgrounds

One of the main target of reactive oxygen species (ROS) are the thiols group of proteins, which upon oxidation are regenerated through activity of thioredoxins. *Leptospirillum* spp. is a bioleaching microorganism that is naturaly exposed to conditions as acidic pH and high concentrations of metals, that contribute to ROS generation and consequently induction of oxidative stress and biomolecule damage. Bioinformatic studies in *Leptospirillum* sp. CF-1 allowed to identify multiple thioredoxin coding genes (*trx)*. However, it is unknown what is the contribution of each *trx* gene to tolerate oxidizing conditions

Objectives

In this research, we analyzed the individual participation of 13 *trx* genes from *Leptospirillum* sp. CF-1 in response to oxidative stress

Methods

Role of the thioredoxins was addressed by using a bioinformatic approach in combination with genetic complementation assays carried out in Escherichia coli, and evaluating mRNA levels of *trx* genes from *Leptospirillum* sp. CF-1 exposed to ferric sulfate and diamide.

Conclusions

Analysis revealed that predicted thioredoxins can be assigned to functional categories including thioredoxins, peroxiredoxins, and disulfide oxidoreductases, among others. The 13 *trx* genes were transcriptionally active, although they responded differentially to ferric sulfate or diamide stress. Moreover, there was one *trx* gene that was fulfilling a more general role responding to both oxidative agents. It was demonstrated that some of these genes conferred oxidative protection to a thioredoxin-deficient strain of *E. coli* by restoring the wild-type phenotype under oxidative stress conditions. These findings can be the basis to understand the adaptations of acidophilic microorganisms to extremely oxidative bioleaching environments, with a possibility for an improvement of the bioleaching industrial process.

FEMS7-1047
Physiology / Biochemistry / Molecular Microbiology - Part II

A PHAGE PROTEIN INTERACTS WITH TYPE IV PILI FOR SUPERINFECTION EXCLUSION

Y. Li[1], X. Wang[1]
[1]Chinese Academy of Sciences- South China Sea Institute of Oceanology, LMB, Guangzhou-, China

Backgrounds

Filamentous prophage Pf4 in *Pseudomonas aeruginosa* PAO1 plays an important role in biofilm development, Pf4 was able to convert into superinfective phage during the biofilm formation of PAO1, and the released Pf4 phages were capable of re-infecting the PAO1 host, The underlying mechanism of Pf4 superinfection remains largely unknown.

Objectives

A small protein PA0721 encoded by Pf4 plays an important role in Pf4 superinfection exclusion.

Methods

Overexpression of PA0721 in the PAO1 and Pf4 deletion mutant strains resulted in the abortion of Pf4 superinfection and the loss of twitching motility. Twitching motility allows *Pseudomonas aeruginosa* to respond to stimuli by extending and retracting its *type IV pili*. PilJ is a protein necessary for this surface-associated twitching motility and bears high sequence identity with the methyl-accepting chemotaxis proteins in *E. coli*. Since the uptake of Pf4 requires the type IV pili, we reasoned that PA0721 might interact with the components of type IV pili to block twitching motility. GFP-fused PA0721 was localized to the cell poles and it interacted with PilJ via the periplasmic domains of PilJ. Furthermore, the minor coat protein PA0724 of Pf4 also interacts with PilJ.

Conclusions

The entry of Pf4 into PAO1 might be mediated by the interaction of PA0724 and PilJ, and PA0721 might disrupt this interaction to inhibit Pf4 superinfection. Additionally, genes encoding PA0721 and PA0724 are conserved among Pf filamentous phages. Therefore, PA0721-mediated inhibition of twitching motility may reveal a common mechanism for the superinfection exclusion of Pf filamentous phages.

FEMS7-2603
Physiology / Biochemistry / Molecular Microbiology - Part II

AN IMPROVED CRIB REPORTER ENABLES THE IDENTIFICATION OF INDIVIDUAL HYPHAE OF TRICHODERMA ATROVIRIDE ENGAGED IN MYCOPARASITIC ATTACK

A. Lichius[1], *L. Salzmann*[1], *D. Moreno-Ruiz*[1], *S. Zeilinger*[1]
[1]*University of Innsbruck, Institute of Microbiology, Innsbruck, Austria*

Backgrounds

Launching a mycoparasitic attack depends on the perception of host-derived signals by individual hyphae of the mycoparasite. However, not all hyphae constituting the colony periphery execute the same cellular programs at the same time. Therefore, a principal difficulty for researching host-parasite interactions on the cellular and molecular level is the identification of individual hyphae that at the time of observation are actively engaged in mycoparasitic signalling. This becomes specifically important for single-molecule imaging techniques where pinpointing such active hyphae is a prerequisite for efficient data acquisition.

Objectives

Our rational was to establish a fluorescently-labelled CRIB reporter to visualise those peripheral hyphae of the mycoparasite *Trichoderma atroviride* that grow positively chemotropic towards a host fungus, and thus most likely execute mycoparasitic cellular programs.

Methods

Fluorescent CRIB reporters exclusively associate with active, i.e. GTP-bound, Cdc42 and Rac1 GTPases, and specifically label hyphae that show active polarised tip growth. Using site-directed mutagenesis we generated an EGFP variant with 20-fold increased brightness which significantly improved CRIB detection. To allow a clear distinction between parasite and host hyphae in the contact zone we used as confrontation partner and prey *Botrytis cinerea* expressing cytoplasmic mCherry.

Conclusions

The novel *T. atroviride* CRIB-mbasicGFP reporter localises inside the Spitzenkörper and as apical crescent/cap in growing hyphae. Repositioning of apical GTPase activity precedes and thus regulates tip growth directionality. Hence, the analysis of CRIB reporter dynamics allows differentiation and prediction of positively chemotropic hyphae, and therefore enables a quantitative approach on mycoparasitic signaling in relation to host-parasite distance and different host species.

FEMS7-0963
Physiology / Biochemistry / Molecular Microbiology - Part II

SPOT 42 BINDING TO DIFFERENT GAL MRNAS RESULTS IN DIFFERENTIAL CONSEQUENCES

H. Lim[1], Y. Lee[1], H.J. Jeon[1], X. Wang[1]
[1]Chungnam National University, Biology, Daejeon, Republic of Korea

Backgrounds

Most of small RNAs (sRNA) in *Escherichia coli* work by binding to target mRNA through base-pairing. Spot 42, a sRNA of 109 nucleotide-long, harbors 3 regions in its 5' half, about 10 nucleotide each for binding to target mRNAs. The *gal* operon has 4 structural genes in *E, T, K* and *M* order and harbors a Spot 42 binding site at *T-K* junction. Out of 6 *gal* mRNAs, 4, mT1, mK2, mK1, and mM1 have the same Spot 42 binding site, thus, in different locations relative to their 3'ends.

Objectives

In this study, we investigated molecular consequences of Spot 42 binding to these *gal* mRNAs.

Methods

3' Race (Rapid Amplification of cDNA End) assay and Northern blot analysis.

Conclusions

We found that Spot 42 binding to mT1 causes mT1 production, but that to mK2, mK1, and mM1 leads to degradation. Spot 42 binds to a transcript between RNA polymerase (RNAP) and leading ribosome (LR), causing 1) RNAP to pause transcription and 2) blocking translation initiation of LR. These two events, respectively, produce the 2 clusters of mT1 3'ends. These results suggested that if Spot 42 binding occurs to emerging transcript, the binding leads to production of target mRNA, but if that occurs to existing transcript, i.e. post-transcriptionally, the binding leads to degradation of target mRNA.

FEMS7-0821
Physiology / Biochemistry / Molecular Microbiology - Part II

FUNCTIONAL ANALYSIS OF INDOLE-3-ACETIC ACID METABOLIC GENES IN ACINETOBACTER BAUMANNII

G.H. Lin[1]
[1]Tzu Chi University, Microbiology, Hualien, Taiwan R.O.C.

Backgrounds

Indole-3- acetic acid (IAA) plays an important role in crop development. Bacteria in rhizosphere such as *Pseudomonas* or *Acinetobacter* evolved with the ability to degrade and product IAA.

Objectives

In order to understand the biological function of IAA metabolic genes in rhizosphere, both of IAA degradation and synthesis genes will be studied in detail.

Methods

Marker-less and insertional method were conducted to product mutants for the study of biological and biochemical of those genes. A modified rapid method employed LC/MS-MS was used to analyze the concentration of IAA was established by our group. Quantitative RT-PCR was carried out to determined transcription of different genes.

Conclusions

The *iac* gene cluster contributes to the regulation and degradation of IAA in *A. baumannii.* IacR, a MarR family regulator represses *iac* operon expression in the absence of IAA. In the presence of IAA, IAA served as a ligand to remove IacR from the promoter and RNA polymerase promote the expression of *iac* operon for IAA degradation. Culture experiment, qRT-PCR and GC/MS-MS results determined that *A. baumannii* produced IAA via indole pyruvate (IpyA) route. In summary, IacR regulates *iac* operon expression which is functions for IAA degradation. IpyA is the major route for IAA production via a tryptophan-dependent pathway. *A. baumannii* manipulates IAA concentration in rhizosphere by equipping production and degradation machinery with differential regulatory mechanisms.

FEMS7-0162
Physiology / Biochemistry / Molecular Microbiology - Part II

THE SELF-IMMUNE RESPONSE OF TRICHODERMA TO ITS OWN SECONDARY METABOLITES

C.M. Liu[1], S.Y. Liu[2], C.K. Liao[1], H.L. Peng[2], N.R. Vaikar[1], K.C. Peng[1]
[1]National Dong Hwa University, Department of Life Science and Institute of Biotechnology, Hualien, Taiwan R.O.C.
[2]Da-Yeh University, Department of Molecular Biotechnology, Changhua, Taiwan R.O.C.

Backgrounds

Alternative approaches, such as biological control, have been employed to manage soil-borne plant pathogens. Several *Trichoderma* spp. have been shown to reduce the severity of plant diseases by inhibiting plant pathogens through their highly potent antagonistic and mycoparasitic activity. Plant growth promotion, defenses and tolerance to biotic & abiotic stress benefit from *Trichoderma* as well. The biocontrol activities are driven by variety of secondary metabolites secreted by *Trichoderma*.

Objectives

Previously we identified several metabolites involved in *Trichoderma*'s biocontrol mechanism, by inducing mitochondria-mediated apoptosis in phytopathogens and lead to deadly lysis in the end. In this study, we further investigate the self-immune response induced by *Trichoderma*'s own metabolites.

Methods

MALDI-TOF and LC-MS/MS analysis identified four significantly up-regulated expressed proteins: HEX1 , nitroreductase, transaldolase, and serine hydroxymethyltransferase. Quantitative PCR analysis indicated the up-regulated expression of corresponding mRNA as well. Apparently certain metabolic pathways and signaling were accelerated to protect *Trichoderma* itself from oxidative burst. Four ROS-related enzyme activities were also measured following 3-day treatment with 30 ppm of specific metabolites. SOD was significantly increased, PEX slightly increased, and GST showed no variation. However, CAT exhibited about 25% decrease instead. The escalated ROS level within *Trichoderma* hyphae seemed scavenged by highly induced SOD.

Conclusions

The highly expressed HEX1, NTR, TRAD, SHMT, and SOD following 3-day treatment with its own secondary metabolites definitely involved in self-immune response of *Trichoderma*. The investigation of specific roles played by these proteins and signaling network within is underway.

FEMS7-0172
Physiology / Biochemistry / Molecular Microbiology - Part II

CABBAGE RESISTANCE AGAINST PHYTOPATHOGENS INVOLVING TRICHODERMA METABOLITES

C.K. Liao[1], S.Y. Liu[2], H.L. Peng[2], K.C. Peng[1]
[1]National Dong Hwa University, Department of Life Science and Institute of Biotechnology, Hualien, Taiwan R.O.C.
[2]Da-Yeh University, Department of Molecular Biotechnology, Changhua, Taiwan R.O.C.

Backgrounds

It is well known *Trichoderma* spp contribute crucial benefits to the host plant through variety of proposed mechanisms such as boosting host plant defense responses against phytopathogens. The underlining mechanism of induced systemic resistance and systemic acquired resistance in primed plants provoked by *Trichoderma* spp remained an interesting issue.

Objectives

Previously, we showed two major *Trichoderma* secondary metabolites, namely chrysophanol and anthraquinone, exhibited significant antimicrobial activities. In this study, we further investigate any beneficial effect of these two compounds to the host plants and their intervention during phytopathogen infection.

Methods

Cabbage was mainly used as the host plant in this study. The seed germination rates were calculated following chrysophanol/anthraquinone treatment. The whole plant development, as well as susceptibility to phytopathogen infections, was assessed following chrysophanol/anthraquinone treatment or *Trichoderma* colonization. Further investigation with q-PCR to learn the variation of pathogenesis related proteins (PR-1), cell wall degrading enzymes, and reactive oxygen species scavenging enzymes were also conducted. The leaf infected area can be reduced significantly either by the presence of *Trichoderma* in rhizosphere or chrysophanol/ anthraquinone treatment prior to phytopathogen challenge. In addition, DNA fragmentation was inhibited, PR-1 suppressed, while GST, SOD, and ascorbate peroxidase induced.

Conclusions

Both compounds at the concentration of 1,000 ppm, better effectiveness of chrysophanol than pahybasin, dramatically reduced the susceptibility of cabbage to phytopathogen infection. Through scavenging cytotoxic reactive electrophiles, induced GST, SOD, and ascorbate peroxidase significantly reduced the oxidative tissue damage in phytopathogen-infected cabbage. Both compounds indeed promoted the defense response of cabbage against phytopathogens tested.

FEMS7-2658
Physiology / Biochemistry / Molecular Microbiology - Part II

FUNCTIONAL AND GENETIC ANALYSIS OF A SYSTEM THAT RAISES THE FITNESS LOCATED ON THE NATIVE BACILLUS SUBTILIS PLASMID PLS20.

A. López Pérez[1], *W. Meijer*[1]
[1]*Centro Biología Molecular Severo Ochoa, Virology and Microbiology, Madrid, Spain*

Backgrounds

The emergence of (multidrug) resistant bacteria (MRB) is one of the main clinical problems today. There are at least three different strategies to combat the increasing problems associated with MRB. First, identifying novel cellular targets for the development of new potential antibiotics, second, identifying and developing new antibiotics or/and new antibiotic-strategies against existing cellular targets, and third, studying the mechanisms responsible for the dissemination of antibiotic resistances and use the information to develop drugs to interfere with these processes

Objectives

Studying the mechanisms responsible for the dissemination of antibiotic resistances and use the information to develop drugs to interfere with these processes

Methods

Bacteria evolve rapidly which is especially due to their ability to exchange genetic material, which can occur via different processes, collectively named Horizontal Gene Transfer (HGT). Most bacteria contain plasmids that can be transferred to plasmid-free bacteria in a process called conjugation. Antibiotic resistance genes are often located on conjugative plasmids. Therefore, plasmid conjugation plays a major role in the spread of antibiotic resistance.

Conclusions

Our results indicate that, besides of conjugation, pLS20 has another mechanism that allows most of the cells in an ecological niche to have the plasmid. We have focused on the functional and genetic analysis of a cassette of two plasmid gene. These genes constitute a functional module that together raise the fitness of *Bacillus subtilis* cells harboring the plasmid.

FEMS7-1922
Physiology / Biochemistry / Molecular Microbiology - Part II

TRANSMEMBRANE REDOX AND PROTEOLYSIS CONTROL OF C-DI-GMP SIGNALING IN BACTERIAL BIOFILM FORMATION

M. Lorkowski[1], S. Herbst[1], O. Sarenko[1], K. Nguyen[1], T. Jaenicke[1], R. Hengge[1]
[1]Humboldt-University of Berlin, Biology/Microbiology, Berlin, Germany

Backgrounds

The nucleotide second messenger c-di-GMP nearly ubiquitously promotes bacterial biofilm formation, with enzymes that synthesize and degrade c-di-GMP being controlled by diverse N-terminal sensor domains.

Objectives

Here we describe and compare members of a novel class of widely occurring c-di-GMP phosphodiesterases that feature a periplasmic 'CSS domain' flanked by two transmembrane regions (TM1/TM2) and followed by a cytoplasmic EAL domain with phosphodiesterase activity.

Methods

Using PdeC of *E. coli* as a model, we show that DsbA/DsbB-promoted disulfide bond formation in the CSS domain inhibits activity. By contrast, the free-thiol form is enzymatically active, which depends on TM2 as a dimerization domain. Moreover, this form is processed by periplasmic proteases DegP and DegQ, yielding an irreversibly activated TM2+EAL fragment slowly removed by further proteolysis.

Conclusions

This versatile interplay of redox control and proteolysis of PdeC controls cellular c-di-GMP and thereby the production of amyloid curli fibres and cellulose, i.e. major biofilm matrix polymers.

FEMS7-2217
Physiology / Biochemistry / Molecular Microbiology - Part II

DISTRIBUTION OF FITNESS EFFECTS OF RANDOM MUTATIONS IN THE SALMONELLA TYPHIMURIUM LT2 GENOME

U. Lustig[1], D.I. Andersson[1]
[1]Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden

Backgrounds

The distribution of fitness effects (DFE) of random mutations for a whole genome is a key determinant of how evolution progresses in response to mutation, selection and drift. Few studies have directly measured the fitness effects of random single mutations in the genome in their native context with high sensitivity. New techniques like whole genome sequencing (WGS) and Magnetic-Activated Cell Sorting (MACS) allow us to study the distribution of mutations and fitness changes in detail.

Objectives

To determine the DFE and identify the types of mutations that are accumulated in the *Salmonella typhimurium* genome under conditions when genetic drift is high.

Methods

We serially passaged *Salmonella typhimurium* LT2 on solid rich media for 2000 generations under conditions expected to result in a high genetic drift (i.e. one cell bottleneck). The 200 evolved lineages were whole genome sequenced (Illumina MiSeq) and analysed for SNPs, insertions, deletions, amplifications and compared to the ancestral parental strain. Fitness measurements were performed by competing the evolved lineages (YFP-labelled) with an isogenic ancestral strain (BFP-labelled), using Magnetic-Activated Cell Sorting (MACS). The selection coefficients were calculated for the evolved lineages and compared to the ratio of the ancestral YFP/BFP strains.

Conclusions

The whole genome sequence data revealed that the majority of the evolved lineages had acquired between 1 and 4 mutations. The main fraction of the mutations were SNVs and 80% were located in coding regions. The fitness data show a distribution of selection coefficients between -0.6809 and 0.0687 for all lineages and an average change in fitness of -0.031.

FEMS7-1867
Physiology / Biochemistry / Molecular Microbiology - Part II

WHEN ANTISENSE MAKES SENSE: EXPLORING THE ROLE OF RNA POLYMERASE-BINDING RNA APTAMERS IN CONTROL OF BACTERIAL ANTISENSE TRANSCRIPTION

A. Magan Garcia[1], N. Sedlyarova[1], R. Schroeder[1]
[1]Max F. Perutz Laboratories, Department of Biochemistry and Cell Biology, Vienna, Austria

Backgrounds

RNA polymerase (RNAP) is the enzyme responsible for transcription in *Escherichia coli*. Its activity is tightly controlled by multiple factors, such as proteins, RNAs and small molecules. We have identified a new class of RNA regulatory elements that modulate transcription in *cis*, either by leading to premature termination or antitermination. RNAP-binding RNA aptamers (RAPs) are relatively short RNA sequences (30-100 nucleotides) binding to the RNAP with high affinity. There are approximately 15,000 RAPs 'encoded' in the *E. coli* genome and the majority (~60%) is found antisense to annotated genes (asRAPs). Since the *E. coli* genome is pervasively transcribed from both strands, we hypothesised that antisense RAPs play a significant role in controlling antisense transcription, both positively and negatively.

Objectives

We studied the potential role of asRAPs in modulating antisense transcription. We particularly focused on how RAPs modulate transcriptional interference (TI) happening from two convergent promoters.

Methods

Differential expression analyses of the *E.* coli transcriptome were performed and several datasets were obtained (i.e. RNA 3' end mapping and RNA deep-sequencing). Combining these data, we were able to identify several asRAP candidates with regulatory potential. The activity of these candidates was studied in depth on TI plasmid-based reporter systems by diverse methods (RT-qPCR, Northern blotting, and fluorescence reporter assays).

Conclusions

Our preliminary data suggest that certain asRAPs are able to terminate transcription from the antisense strand, reducing TI and increasing the expression level of genes expressed from the sense promoter. We therefore suggest that asRAPs are widespread modulators of transcription interference.

FEMS7-2125
Physiology / Biochemistry / Molecular Microbiology - Part II

MUTATIONS IN GENES OF 3R SYSTEM OF NEISSERIA GONORRHOEAE AS THE CAUSE OF THE 1901ST CLONE SUCCESS

N. Bodoev Ivan[1], M. Malakhova[1], I. Alexander Manolov[1], I. Karpova[2], E. Kostryukova[2], E. Ilina[1], M. Vadim Govoruna[1]
[1]Federal Research and Clinical Center of Physical-Chemical Medicine, Laboratory of Molecular microbial genetics, Moscow, Russia
[2]Federal Research and Clinical Center of Physical-Chemical Medicine, Laboratory of Postgenomic Research in Biology, Moscow, Russia

Backgrounds. The expansion of drug resistant bacteria is one of the global contemporary problems. *N. gonorrhoeae* (NG) is a gram-negative bacterium which causes common sexually transmitted diseases gonorrhea and easy acquires various mechanisms of drug resistance (DR). Recent studies of DR evolution were considered in the context of the high rates of recombination within the *Neisseria* genus. It was shown that NG strains of sequence type 1901ST are very successful in DR developing and spreading. However, not all of 1901ST strains bring the known DR determinants.

Objectives. In this work we tried to establish the relationship between the unique set of mutations in 3R (Repair, Replication, and Recombination) system genes of 1901ST strains and their successfulness.

Methods.

83 NG strains with known susceptibility to the cefixime, azithromycin and tetracycline including both our 1901ST strains NG51 and NG0705 and whole genome sequenced strains from the NCBI database were selected for the cluster analysis based on the 3R system genes polymorphism. Mutation rates in NG51 and NG0108 (ST 12443) strains were estimated by fluctuation test.

Conclusions.

It was shown that 1901ST strains form a distinct phylogenetic cluster on the tree built on a set of 3R system gene polymorphism. It could assume higher mutation rates in successful 1901ST NG strains; however, this assumption was not confirmed by the results of the fluctuation test.

FEMS7-1111
Physiology / Biochemistry / Molecular Microbiology - Part II

HOW CAN TYPE IV SECRETION SYSTEM SECRETE INTEGRAL MEMBRANE PROTEINS?

S. Malmsheimer[1], *L. Krampen*[1], *S. Wagner*[1]
[1]*Institut für Medizinische Mikrobiologie und Hygiene, Sektion Zelluläre & Molekulare Mikrobiologie, Tübingen, Germany*

Backgrounds

During the course of infection, *L. pneumophila* and *C. burnetii* translocate a large number of effector proteins in a type IV secretion system (T4SS)-dependent manner into the host cell to promote intracellular survival and colonization. Among these effector proteins are integral membrane proteins that find their final destination in one of the host cell's membranes. Transmembrane domains (TMDs) within T4SS substrates create a targeting conflict within the secreting bacterium because signals for two different incompatible secretion pathways are interlinked in the same protein: Transmembrane helices are by themselves a membrane targeting and integration signal while the C-terminal translocation signal destines proteins for secretion through T4SSs.

Objectives

We aim to understand how the bacterial cell discriminates between inner membrane targeting and secretion by the T4SS.

Methods

We studied the membrane targeting and integration potential of four out of 90 TMD-containing T4SS substrates of *Legionella pneumophila* and *Coxiella burnetii* in different *in vivo* model systems.

Conclusions

We noticed that some proteins avoid inner membrane insertion by a fine-tuned lower hydrophobicity of their TMDs, which is similar to what we observed for T3SS substrates in *Salmonella Typhimurium*. Other T4SS substrates, however, show a markedly high hydrophobicity of their TMDs. While these effectors have been reported to be *bona fide* T4SS substrates, we observe that their TMDs are able to promote inner membrane insertion. Either these substrates take a novel T4SS pathway that involves inner membrane intermediates or a so far unknown mechanism exists that prevents futile membrane targeting of these T4SS substrates in *Legionella* and *Coxiella*.

FEMS7-2986
Physiology / Biochemistry / Molecular Microbiology - Part II

EVOLUTION OF GENE CLUSTERS IN FUNGI

M. Marcet-Houben[1], T. Gabaldón[1]
[1]Center for Genomic Regulation, Bioinformatics and Genomics, Barcelona, Spain

Backgrounds

Although to a lesser extent than in prokaryotes, eukaryotes also show some level of conservation of gene order among functionally related genes. An illustrating example are the gene clusters responsible for the synthesis of secondary metabolites in fungi. Such clusters are present across different fungal genomes, often showing sparse distributions and being present in very divergent species.

Objectives

We aim to detect gene clusters conserved among divergent fungal species and asses which evolutionary processes drive such distributions.

Methods

We developed a new method to detect gene clusters across genomes based on a comparative genomics approach. We used this method on 280 diverse fungal species and detected 189,000 gene clusters, which could be grouped in 25,689 families. Based on a phylogenomics approach we established the most likely evolutionary scenario for the formation of the clusters involving several mechanisms such as vertical evolution, horizontal gene transfer, or convergent evolution.

Conclusions

We established that most cluster families present in divergent species owed their presence to the independent formation of the cluster (71% of the cases). Surprisingly horizontal gene transfer events of whole gene clusters seem to play only a secondary role in the formation of cluster families (1.3% of the cases).
This study provides a novel methodology to detect gene order conservation and answers the question on how gene clusters in fungi have evolved. Yet it opens new questions on the mechanisms that allow the same cluster to be formed multiple times in divergent fungal species.

FEMS7-1980
Physiology / Biochemistry / Molecular Microbiology - Part II

RELEVANCE OF MIXED FUNGEMIA BY CANDIDA PARAPSILOSIS AND CANDIDA ORTHOPSILOSIS

C. Marcos Arias[1], E. Cantón[2], N. Pena-Fernández[1], E. Mateo[1], J. Pemán[2], E. Eraso[1], G. Quindós[1]
[1]Universidad del País Vasco UPV/EHU, Inmunología- Microbiología y Parasitología, Bilbao, Spain
[2]Hospital Universitari i Politécnic La Fe, Servicio de Microbiología, Valencia, Spain

Backgrounds

Incidence of *Candida parapsilosis* invasive candidiasis is increasing. However, *Candida parapsilosis* is a complex of cryptic species phenotipically undistinguishable that hinders the diagnosis.

Objectives

To evaluate the incidence of mixed fungemia by *Candida parapsilosis* and *Candida orthopsilosis*.

Methods

Identification of 96 *Candida parapsilosis* complex clinical isolates from 93 patients suffering candidaemia at the Hospital Universitario y Politécnico La Fe (Valencia, Spain) was performed by two RFLP-PCR methods. Restriction patterns of the secondary alcohol dehydrogenase gene (*SADH*) and *FKS1* gene were analyzed in a complementary way. When both methods failed to reach an agreement on the species identity, analysis of the D1/D2 domain of 26S LSU gene sequence was performed.

Conclusions

Seventy eight isolates out of 96 of *Candida parapsilosis sensu stricto* (81.2%), 15 of *Candida orthopsilosis* (15.6%) and three of *Candida metapsilosis* (3.1%) were identified. Mixed cultures were observed in Candida Chromogenic Agar (Laboratorios Conda, España) from 3 out of 93 patients (3.2%): the presence of *Candida parapsilosis sensu stricto* and *Candida orthopsilosis* in the same blood specimen was confirmed by molecular methods. Concordance between the two RFLP-PCR methods was good (89.6%), although the method involving *SADH* gene misidentified four isolates (4.2%).

Candida orthopsilosis, *Candida parapsilosis* and mixed *Candida parapsilosis* complex candidaemias are not uncommon and careful selection of molecular identification methods is recommended.

Funding: Fundación ONCE and projects GIC15/78 IT990-16 (Gobierno Vasco/Eusko Jaurlaritza) and UFI 11/25 (UPV/EHU).

FEMS7-1767
Physiology / Biochemistry / Molecular Microbiology - Part II

A FASCINATING EXAMPLE OF CONVERGENT EVOLUTION INVOLVES STAPHYLOCOCCUS AUREUS PHAGE ENCODED DIMERIC AND TRIMERIC DUTPASES IN SIGNALLING

J. Donderis[1], E. Maiques[1], J.Z. Bowring[2], J.R. Ciges[1], M.M. Neamah[2], C. Alite[1], M.A. Tormo-Mas[3], J.R. Penadés[2]
[1]Institute of Biomedicine of Valencia CSIC, Genomic and Proteomic, valencia, Spain
[2]University of Glasgow, Institute of Infection- Immunity and Inflammation., Glasgow, United Kingdom
[3]Instituto Investigación Sanitaria La Fe, Grupo Infección Grave, Valencia, Spain

Backgrounds

The dUTPase (Dut) enzymes prevent the misincorporation of uracil into the DNA and are encoded by almost all free-living organisms and some viruses. We have previously showed that phage-encoded trimeric Duts mediates the *Staphylococcus aureus* pathogenicity island (SaPIs) transfer by interacting to the SaPI-encoded repressor Stl, proposing that these Duts are regulatory proteins. Some S. aureus phages encode structurally unrelated dimeric Duts instead trimeric Duts. Surprisingly, a recent work, has involved one of these predicted dimeric Duts in the transfer of SaPIs by interacting with the same Stl repressor.

Objectives

To analyse the molecular basis of SaPI induction by dimeric Duts and to compare with the mechanism reported for trimeric Duts.

Methods

Using sequence analysis we have divide S. aureus phage-encoded Duts in six families. The SaPI mobilization capacity of one representative dimeric Dut from each family was examined *in vivo* as well as its binding capacity to Stl repressor *in vitro*. We analysed the effect of the dUTP in the Stl-Dut interaction. Finally, we solved the 3-D structure by X-ray crystallography of several dimeric Duts in different activation states

Conclusions

The results that will be presented in the communication show that dimeric and trimeric Duts from *S. aureus* phages present a striking parallelism in the mechanism of SaPI mobilization. These similarities would confirm the role of dUTP as new nucleotide with second messenger function. However, some differences suggest peculiarities in the molecular mechanism of Stl recognition and binding for each type of Duts. Our results support the idea that the signalling role of the Dut proteins is an important force driving evolution and speciation

FEMS7-1017
Physiology / Biochemistry / Molecular Microbiology - Part II

THE TWO AAA+ ENHANCER-BINDING PROTEINS (BEBPS) REDR1 AND REDR2 FORM HETEROHEXAMERS TO ACTIVATE TRANSCRIPTION OF AZOARCUS ANAEROBIUS 1,3-DIHYDROXYBENZENE ANAEROBIC DEGRADATION PATHWAY

D. Pacheco[1], *P. Marín*[1], *M. Grifé-Ruíz*[1], *S. Marqués*[1]
[1]*Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas, Environmental Protection, Granada, Spain*

Backgrounds

The two homologous bacterial AAA+ enhancer-binding proteins RedR1 and RedR2 of the obligate anaerobe *Azoarcus anaerobius* activate transcription at three promoters controlling the anaerobic degradation of 1,3-dihydroxybenzene (1,3DHB, resorcinol). Despite 97% identity between the two bEBPs, they are controlled by different activation mechanisms: RedR1 becomes fully active when repression by its N-terminal domain (NTD) is released after effector binding (negative control), whilst RedR2 requires its NTD to be constitutively active (positive control) and is kept inactive in the cell through binding to the membrane protein BtdS.

Objectives

The two bEBPs are required for full activity of the promoters, and bEBPs must oligomerize into hexamers to activate transcription. The aim of this work was to analyze the promoters driving expression of the three operons and to determine if the two regulators were able to form heterohexamers. The activation mechanism of each regulator was further analyzed.

Methods

Transcription analysis, bacterial adenylate cyclase two-hybrid (BACTH) assays, protein pull-down and site-directed mutagenesis were used to identify the interactions and analyze the activation mechanisms.

Conclusions

Only P_{orf14} promoter was active in the heterologous host *E. coli*. Transcription activation required the active form of either RedR1 (the NTD-truncated protein) or RedR2 (the whole-length regulator); the promoter was strictly dependent on σ^{54} and IHF. BACTH assays showed interaction between RedR1 and RedR2 central domain, partially stabilized by their C-terminal domain. No self-interaction of either regulator was observed. This interaction pattern was confirmed in pull-down experiments. We conclude that redR1 and RedR2 form hetero-hexamers to activate transcription of the pathways. The RedR2 residues involved in the interaction with BtdS have been identified.

FEMS7-0452
Physiology / Biochemistry / Molecular Microbiology - Part II

LIKE THE AIR WE BREATHE: THE ROLE OF UROPATHOGENIC ESCHERICHIA COLI TERMINAL REDUCTASES DURING INFECTION IN VITRO AND IN VIVO

A.J. Martín-Rodríguez[1], *K. Melican*[1], *A. Richter-Dahlfors*[1]
[1]*Karolinska Institutet, Swedish Medical Nanoscience Center, Stockholm, Sweden*

Backgrounds

Urinary tract infections follow an ascending path from the urethra to the bladder (cystitis) and the kidneys (pyelonephritis). The urinary tract is a moderately oxygenated environment in which a variety of niches exist. For instance, the oxygen concentration in the bladder is reported to be around 4-7 %, whereas local fully anoxic conditions have been found in the renal tubule lumen during bacterial infection.

Objectives

Our goal is to explore the role of UPEC anaerobic respiration during host colonization and the expression of infection-relevant phenotypes. Specifically, we aim to understand the contribution of individual respiratory terminal reductases to an array of infection phenotypes *in vitro* and *in vivo*.

Methods

In this work, which is presented as part of ongoing research, we use *E. coli* CFT073 as a protoypic uropathogenic *Escherichia coli* (UPEC) strain. Using lambda red-mediated recombineering, we have systematically knocked out all the operons encoding anaerobic terminal reductases in this strain, as well as *fnr* whose gene product, the global transcriptional regulator FNR, is required for the switch from aerobic to anaerobic metabolism in *E*. *coli*. We are analyzing the effect of these deletions on biofilm formation, motility, antimicrobial susceptibility and *in vitro* infection. Subsequently, we aim to move beyond the bench and validate our findings using ascending infection and intravital animal models.

Conclusions

Our results shed light on the role of alternative electron acceptors as key fitness factors for UPEC infection, including the modulation of virulence-relevant phenotypes.

FEMS7-1276
Physiology / Biochemistry / Molecular Microbiology - Part II

AMRZ IS A GLOBAL ENVIRONMENTAL RESPONSE REGULATOR INVOLVE IN C-DIGMP TURNOVER IN PSEUDOMONAS FLUORESCENS F113

M. Martín[1], C. Muriel[1], E. Arrebola[1], F. Martínez-Granero[1], E. Blanco-Romero[1], D. Garrido-Sanz[1], M. Redondo-Nieto[1], R. Rivilla[1]
[1]Universidad Autónoma de Madrid, Biología, Madrid, Spain

Backgrounds

AmrZ, alginate and motility regulator Z, is a regulator protein belonging to the Arc family that contains a Ribbon-Helix-Helix (RHH) DNA binding domain. AmrZ is a master regulator of gene expression across Pseudomonads. The expression of *amrZ* depends on the environmental stress sigma factor AlgU which itself has been implicated in alginate production, regulation of virulent factors and adaption traits. *Pseudomonas fluorescens* F113 is a Plant Growth Promoting Rhizobacteria (PGPR) which has been considered as a model strain for rhizosphere colonization studies. In this strain, AmrZ regulates swimming motility through the repression of FleQ.

Objectives

To investigate the c-diGMP related regulon of AmrZ in *P. fluorescens* F113

Methods

A ChIP seq analysis of AmrZ in P. *fluorescens* F113 revealed that this protein is involve in environmental sensing and adaption.

Conclusions

AmrZ regulates not only the motility but also the iron uptake, the cell wall maintenance and the virulence among other traits. However, most of these phenotypes are influenced by the AmrZ dependent changes in c-diGMP. In this work we have analyze the phenotype of 15 mutants in genes codifying phosphodiesterases and diguanylate cyclases regulated by AmrZ. The implication of these enzymes in *P. fluorescens* F113 ability to form biofilms will be discussed.

CM was granted by FPI fellowship program (BES-2013-063189). EBR is the recipient of a fellowship from Fundación Tatiana Pérez de Guzmán el Bueno, Spain. DGS was granted by FPU fellowship program (FPU14/03965) from Ministerio de Educación, Cultura y Deporte, Spain. Funded by BIO 2015-64480-R (MINECO/FEDER EU)

FEMS7-1986
Physiology / Biochemistry / Molecular Microbiology - Part II

UBIQUITINATION: IMPLICATIONS IN THE REGULATION OF GENE EXPRESSION THROUGH THE CWI PATHWAY IN YEAST

R. García[1], V. Mascaraque[1], A.B. Sanz[1], S. Díez-Muñiz[1], C. Nombela[1], J.M. Rodríguez-Peña[1], J. Arroyo[1]
[1]Facultad de Farmacia. Universidad Complutense de Madrid and Instituto Ramón y Cajal de Investiga ciones Sanitarias, Departamento de Microbiología II, Madrid, Spain

Backgrounds

Signal transduction pathways mediated by MAPKs play an essential role in cellular homeostasis and survival. In *Saccharomyces cerevisiae*, stress conditions perturbing cell wall integrity trigger the activation of the cell wall integrity MAPK pathway (CWI) whose result is a transcriptional response controlled by the MAPK Slt2 and the transcription factor Rlm1. In the last years, ubiquitination system has emerged as an important regulator of signal transduction pathways.

Objectives

It has consisted of the identification and functional characterization of elements of the ubiquitination/deubiquitination machinery potentially involved in the regulation of the gene expression through the CWI pathway.

Methods

Ubiquitin-ligases and deubiquitinating enzymes related to cell wall stress (Congo Red and Zymolyase) were identified using phenotypic and transcriptional studies. Chromatin immunoprecipitation (ChIP) was used to study ubiquitination/deubiquitination of promoters and ORFs of CWI-responsive genes under different growth conditions and yeast mutant backgrounds.

Conclusions

Ubiquitin-ligases Rad6, Dia2, Slx8, Mms1, Pep3, Rcy1, Ubr2 and deubiquitinating enzymes Ubp3 and Ubp8 were involved in the regulation of expression of CWI-responsive genes (*MLP1/KDX1, SRL3, PRM5, CWP1*) under cell wall stress. Additionally mutants *dia2*Δ, *slx8*Δ, *mms1*Δ, *pep3*Δ, *rcy1*Δ and *ubp3*Δ were sensitive to Congo Red and/or Zymolyase. Ubiquitination in the ORF of CWI-responsive genes described above was observed as well as a dependence on Dia2, Rad6, Slt2 and Rlm1 in this process. Moreover, Rad6 bound to the ORF of MLP1. These results point to ubiquitination as an additional regulatory mechanism involved in the response to cell wall stress in yeast.

FEMS7-1886
Physiology / Biochemistry / Molecular Microbiology - Part II

EXPRESSION OF THE GLOBAL REGULATOR CSRA IS REGULATED BY THE CPXRA TWO-COMPONENT SYSTEM IN E. COLI

C. Masson[1], *L. VAN MELDEREN*[1]
[1]*Free University of Brussels ULB, département de biologie moleculaire, Gosselies, Belgium*

Backgrounds

Global regulators play major roles in response to a variety of intracellular and extracellular signals, allowing bacteria to adapt to environmental changes. Lack of global regulators generally leads to strong defects. We are interested in CsrA, a post-transcriptional global regulator acting by binding target mRNAs and modulating their translation. This regulator is important for cell growth and regulates central carbon metabolism and social behavior pathways.

Transcriptional regulation of *csrA* expression remains largely unknown although it was shown that Sigma70 and RpoS directly regulate *csrA* transcription.

Objectives

The objective is to identify two-component system (TCS) involved in regulation of *csrA* expression.

Methods

Transcriptional fusion was used in different mutant strains to identify *csrA* regulators. Confirmation and characterization of the *csrA* regulation by *cpxRA* were obtained by western blot, mobility shift assays and footprint experiments

Conclusions

We focus our study on the *cpxRA* system, a TCS sensing periplasm and inner membrane stresses through various signals. *cpxRA* regulate pathways notably involved in envelop repair and homeostasis. Our results show that *csrA* promoter activity is dependent on CpxRA. Phosphorylation state of CpxR is important for *csrA* regulation *in vivo*. We showed that acetyl-phosphate plays an important role in the CpxRA-dependent regulation of *csrA* expression by phosphorylating CpxR independently of CpxA, and that CpxA phosphatase activity counteracted it thanks to its phosphatase activity. Finally, we defined the location and sequence of the two CpxR binding sites on the *csrA* promoter region.

FEMS7-3173
Physiology / Biochemistry / Molecular Microbiology - Part II

SPIROPLASMA POULSONII AS A NEW MODEL FOR ENDOSYMBIONT GENETICS

F. Masson[1], F. Schüpfer[1], B. Lemaitre[1]
[1]EPFL, EPFL-SV-GHI-UPLEM, Lausanne, Switzerland

Backgrounds

Spiroplasma are widespread arthropod-associated bacteria. They exhibit a wide range of interactions with their hosts, from parasitism to mutualism. All species are helical, motile, and do not possess a cell wall, which makes them outstanding with regard to their interaction with other organisms. Some species, like *Spiroplasma citri*, are pathogens with a cyclic lifestyle between an insect host and another environment (plants). Other species are insect endosymbionts: they thrive in the insect body cavity and are transmitted from females to offspring, often during oogenesis. This is the case of *Spiroplasma poulsonii* that infects *Drosophila melanogaster*, causing variety of phenotypes.

Objectives

Pathogenic *Spiroplasma* species have generally been cultivable *in vitro* for a long time, and most of them can be readily transformed. Endosymbiotic *Spiroplasma* however do not develop in general *Spiroplasma* media. Furthermore, they are fragile and unable to perform homologous recombination, which stalls most of the mainstream genomic modification techniques. The bacterial side of their interaction with their host has thus never been approached and required the development of new ways to cultivate and modify them.

Methods

By using a step-by-step optimization process, we developed a cell-free medium where *S. poulsonii* can be maintained for several months out of its host. We then set-up integrative plasmids to genetically modify the endosymbiont genome *in vitro* and to re-implant it in the host.

Conclusions

These technical breakthroughs make *S. poulsonii* one of the very few genetically tractable endosymbionts, paving the route for functional studies of the bacterial determinants of its interaction with its host.

FEMS7-1823
Physiology / Biochemistry / Molecular Microbiology - Part II

HIGH MOLECULAR WEIGHT PROTEINS FROM L. RHAMNOSUS GG MODULATE PRO-INFLAMMATORY RESPONSES IN VITRO

M. Mata Forsberg[1], S. Björkander[1], M. Ndi[2], E. Sverremark-Ekström[1]
[1]Stockholm University, Department of Molecular Biosciences- The Wenner-Gren Institute, Stockholm, Sweden
[2]Stockholm University, Department of Biochemistry and Biophysics, Stockholm, Sweden

Backgrounds

Probiotic lactobacilli reduce the risk of immune related diseases by strengthening the gut epithelium, outcompeting pathogens and modulating the immune system. We, and others, have shown that early-life lactobacilli colonization correlates with a reduced risk to develop allergy. Recently we demonstrated that lactobacilli cell free supernatants (CFS) contain factors able to dampen *Staphylococcus aureus* (*S. aureus*) induced T-cell proliferation and pro-inflammatory cytokine production *in vitro*.

Objectives

To investigate how secreted soluble factors from *Lactobacillus rhamnosus* GG (LGG) modulate immune cell activation and to identify specific factors with the capacity to regulate pro-inflammatory immune responses.

Methods

Human peripheral blood mononuclear cells were stimulated in the presence or absence of whole or size fractionated LGG-CFS. Spin column fractionation generated fractions ranging from 3 to 100 kDa in size and the >100 kDa fraction was further fractionated using high performance liquid chromatography (HPLC). Flow cytometry was used to evaluate cell proliferation and ELISA was used to quantify cytokines in cell culture supernatants.

Conclusions

We conclude that LGG secretes factors of low molecular weights that dampen interferon-γ (IFN-γ) production as well as high molecular weight factors that dampen both IFN-γ and interleukin-17A (IL-17A). The HPLC analysis revealed the presence of one or several heat sensitive proteins with IFN-γ and IL-17A dampening activity. With future sequencing analysis we intend to further describe the characteristics of these proteins.

FEMS7-2284
Physiology / Biochemistry / Molecular Microbiology - Part II

THE ROLE OF INDOLE IN ANTIMICROBIAL RESISTANCE AND BACTERIAL PERSISTER FORMATION IN ESCHERICHIA COLI.

M. Matuszewska[1], S. Forrest[1], S. David[1]
[1]University of Cambridge, Department of Genetics, Cambridge, United Kingdom

Backgrounds

Indole is a widespread biological signalling molecule that affects a range of cellular processes in *Escherichia coli* including plasmid stability, extracellular signalling, virulence control and biofilm formation. Recently, indole has been shown to be involved in the formation of antibiotic persisters as well as being a key player in antimicrobial resistance through upregulation of drug efflux pumps.

Objectives

The aim of this study was to further investigate these two aspects of antibiotic resistance. Wild-type and indole-deficient (ΔtnaA; tryptophanase knockout) strains of *E. coli* BW25113 were compared to determine the influence of indole on the minimum inhibitory concentration (MIC) of a range of antibiotics in clinical use

Methods

Both strains were treated with the same antibiotics at 100x MIC and persister cells were enumerated. Pre-treatment and supplementation of the culture with exogenous indole were used in an attempt to repair the effect of the tryptophanase knockout.

Conclusions

A difference in MIC between the strains was observed for a variety of antibiotics, including Aztreonam, Ceftriaxone and Phosphomycin; indicating a role for indole in resistance. For persister formation the results were more complex. During the exponential phase of growth wild-type cultures gave rise to a higher frequency of persisters than tryptophanase knockout cultures. Stationary phase cultures behaved differently, showing a higher frequency of persisters for the tryptophanase mutant. However, colonies formed by persisters derived from the tryptophanase mutant grew more slowly than wild-type persisters after the antibiotic was withdrawn.

FEMS7-1975
Physiology / Biochemistry / Molecular Microbiology - Part II

THE INFLUENCE OF CULTIVATION MEDIUM COMPOSITION ON ESCHERICHIA COLI BIOFILM FORMATION ON NANOCRYSTALLINE DIAMOND SURFACE

P. Matyska Liskova[1], J. Budil[1], J. Beranova[1], R. Fiser[1], O. Romanyuk[2], E. Ukraintsev[2], A. Kromka[2], I. Konopasek[1]
[1]Charles University- Faculty of Science, Dpt of Genetics and Microbiology, Prague 2, Czech Republic
[2]Institute of Physics of the Czech Academy of Sciences, Department of Optical Materials, Prague 6, Czech Republic

Backgrounds

Nanocrystalline diamonds (NCD) belongs to the materials recently widely investigated for antibacterial potential. Published studies include both bacteria survival tests in suspension with NCD nanoparticles and investigation of the antibacterial potential of NCD films on the surfaces. As NCD films seems to exhibit good biocompatibility with tissue cultures, the NCD based surfaces might be promising for bacterial biofilm growth restriction. Further studies must be performed in order to trace the conditions under which NCD limits the growth of bacterial biofilm.

Objectives

We employed NCD deposited on glass with various termination (H-NCD, O-NCD, F-NCD) and *E. coli* as a representative biofilm producer for testing the ability of bacteria to adhere and to establish and mature the biofilm. We analyzed the conditioning film that develops on the surfaces in different cultivation environments and which can substantially influence the consequential biofilm growth.

Methods

E. coli culture was grown in complex LB and mineral M9 media as a batch culture at low rotation rate in presence of tested material. The resulting biofilm quantified using crystal violet assay. We compared the biofilm mass formed in complex and mineral medium.

Conclusions

We discuss the biofilm growth produced by *E. coli* on glass and glass covered with NCD films. The ability of *E. coli* to form biofilm was shown to depend on the NCD surface treatment, its roughness and chemical composition. We also observed the influence of cultivation medium composition which is directly related to the the conditioning film formation which mediates the further bacterial attachment.

FEMS7-0302
Physiology / Biochemistry / Molecular Microbiology - Part II

SECONDARY GENOME PARA PROTEINS OF DEINOCOCCUS RADIODURANS ARE FUNCTIONALLY SIMILAR AND INTERACT TO EACH OTHER

G. Maurya[1], S. Kota[2], H.S. Misra[1]
[1]BHABHA ATOMIC RESEARCH CENTRE- HBNI- Mumbai, Molecular Biology Division- Life sciences, Mumbai, India
[2]BHABHA ATOMIC RESEARCH CENTRE- Mumbai, Molecular Biology Division, Mumbai, India

Backgrounds

Deinococcus radiodurans, a radiation-resistant bacterium, shows ploidy with four genome replicons viz. chromosome I, chromosome II, a megaplasmid and a small plasmid. The genome is annotated with putative *parA-parB* operons. Except small plasmid, other replicons have their own ParA and ParB proteins. The presence of multiple sets of 'Par' proteins in bacteria harboring multipartite genome system, raises the question on their possible functional redundancy and roles in genome segregation.

Objectives

Functional characterization of ParA2 and ParA3 proteins encoded on chromosome II and megaplasmid in *D. radiodurans,* respectively.

Methods

ParA2 and ParA3 proteins were purified and their interaction with DNA was monitored in absence and presence of ADP, ATP or ATPγS using electro-mobility shift assay and fluorescence anisotropy. Polymerization of both proteins in absence and presence of DNA and nucleotides was monitored using sedimentation assay and light scattering. ATP binding and hydrolysis were compared using [α^{32}P]-ATP and fluorimetry. The bacterial two-hybrid system used for interaction in synthetic *E. coli*. Effects of ParA2 and ParA3 mutation on growth and genome maintenance were studied in *D. radiodurans*.

Conclusions

Both ParA2 and ParA3 bind to DNA and polymerize in similar fashion. These activities improved further in the presence of ATP and both are ATPase *in vitro*. Interestingly, both proteins interact to self as well as each other and their mutant can complement the function of each other in *D. radiodurans* indicating functional redundancy among both proteins.

FEMS7-0767
Physiology / Biochemistry / Molecular Microbiology - Part II

MOLECULAR TYPING OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS COMING FROM SCREENING OF MULTI-RESISTANT BACTERIA OF VERONA, ITALY

A. Mazzariol[1], *L. Galia*[1], *M. Ligozzi*[1]
[1]*University of Verona, Diagnostic an Public Health- Microbiology section, Verona, Italy*

Backgrounds

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-and communities acquired infections

Objectives

The aim of the study was to characterize MRSA isolated during the screening of multi-resistant bacteria and check if there is a prevalent clone.

Methods

A total of 110 *S. aureus* isolates originating from patients at hospital admission in high risk wards were obtained from throat

MRSAs were confirmed by detection of the *mecA* gene by PCR that also included primers to detect *spa, scn, lukS-PV* (PVL) and *mecC.*

spa and SCC*mec* typing was performed by Sanger sequencing and the multiplex PCR protocol by Kondo et. al., respectively

All 110 strains of MRSA were *mecA* positive. 8 strains resulted SCCmec type I (7,27%), 102 are type IV (92,7%) and all 110 strains of class B.

Only 6 strains are positive for *pvl* gene and belong to SCCmec IV.

The 6 *spa type* "representative" of 110 MRSA strains have these results: 38% Spa t032 CC22 (clonal complex), 14% Spa t1036 CC22; 8% Spa t1214 CC22; 7% Spa t022; 7% Spa t041 CC5; 5,4% CC8 Spa t121 (*pvl*-positive).

The t032-IV type resulted the most frequent and belong to the clone type EMRSA15.

Conclusions

92,7% of the strains are SCCmec type IV normally associated with CA-MRSA. Only 7,27% are SCCmec type I normally associated with strains HA-MRSA. The 5,4% of cases are PVL positive.

EMRSA-15 clone has been detected in this study belonging to type more frequently t032-IV CC22.

FEMS7-1840
Physiology / Biochemistry / Molecular Microbiology - Part II

MRNA HOMEOSTASIS IS MAINTAINED AS CELL VOLUME CHANGES IN SACCHAROMYCES CEREVISIAE

A. Mena[1], D.A. Medina[1], J. Garcia-martinez[1], G. Ayala[2], J.E. Perez-ortin[1]
[1]University of Valencia, Biochemistry and Molecular Biology, Burjassot, Spain
[2]University of Valencia, Stadistics and Operations Research, Burjassot, Spain

Backgrounds

Different cell size is found among *Saccharomyces cerevisiae* yeast strains depending on their ploidy or on gene mutations that increase or decrease it with regard to wild type median size and also along the cell cycle, size increases up to a maximum size in mitosis and then abruptly the volume splits into two cells. Thus, control of the mRNA concentrations is required to keep cell homeostasis. Although there is a compartmentalization of mRNA synthesis and degradation activities, a cross-talk between both has been proposed

Objectives

In order to determine if volume influences transcription and mRNA stability in *S. cerevisiae* we analyzed several mutant strains and BY4741 isogenic diploid, triploid and tetraploid strains

Methods

To asses our hypothesis, poly-A signal was measured by dotblot and flux cytometry and poly-A mRNA stability for each population was obtained by transcriptional shut-off and dot blot hybridization. Moreover Genomic-Run-On was performed to obtain RNA nascent transcription.

Conclusions

We found that total RNA (rRNA and tRNA mainly) and mRNA concentrations maintain homeostasis but whereas total nascent transcription (RNA pol I+III mainly) scales with volume, the mRNA turnover (both mRNA synthesis and degradation) is reduced. This last result is also seen in a meta-analysis of the mutant datasets from other group

FEMS7-2183
Physiology / Biochemistry / Molecular Microbiology - Part II

3'UTRS: THE NEW POST-TRANSCRIPTIONAL REGULATORS IN BACTERIA

P. Menendez-Gil[1], *C. Caballero*[1], *A. Toledo-Arana*[1]
[1]*Instituto de Agrobiotecnología, Laboratory of Bacterial Gene Regulation, Mutilva, Spain*

Backgrounds

A messenger RNA comprises a coding sequence flanked by two untranslated regions (UTRs), the 5'UTR (from the transcription initiation to the start codon) and the 3'UTR (from the stop codon to the transcriptional terminator). In eukaryotes, 3'UTRs are essential elements for modulating protein translation by interacting with RNA-binding proteins and microRNAs. Dysregulation of genes whose 3'UTRs suffer mutations or shortening can lead to diseases such as cancer. Recent studies showed that bacterial 3'UTRs are also involved in regulatory functions by controlling biofilm formation, virulence or iron-homeostasis. Moreover, genome-wide transcriptomic analyses revealed that bacteria express hundreds of long 3'UTRs for which a specific role has not yet been discovered.

Objectives

We aimed to perform a high-throughput functional study of bacterial 3'UTRs to decipher new mechanisms driven by these non-coding regions.

Methods

Therefore, we analyzed the transcriptomes of several *Staphylococcal* species that were phylogenetically associated to understand the conservation of 3'UTRs. In addition, we constructed chimeras of genes lacking their 3'UTR or carrying the 3'UTRs of other species and tested how different 3'UTRs affect the protein expression of the same gene.

Conclusions

Transcriptomes showed that one third of *S. aureus* genes contain 3'UTRs longer than 100-nt. However, we found that 3'UTRs are not conserved among *Staphylococcal* species. Western-blot analysis from different chimeras showed that lacking of 3'UTRs changes protein expression, indicating the presence of regulatory elements, and that protein expression differs when carrying different 3'UTRs of the same gene. These results suggest that the post-transcriptional regulation mediated by 3'UTRs has differentially evolved among bacterial species.

FEMS7-2840
Physiology / Biochemistry / Molecular Microbiology - Part II

WHEN SUB-INHIBITORY CONCENTRATIONS OF ANTIBIOTICS PROMOTE THE DISSEMINATION OF UNSELECTED RESISTANCE GENES

H. Scornec[1], H. Guilloteau[1], G. Groshenry[1], C. Pradalier[2], X. Bellanger[1], C. Merlin[1]
[1]CNRS / Université de Lorraine, LCPME, Vandoeuvre-les-Nancy, France
[2]UMI 2958 GT-CNRS, Georgia Tech-Lorraine, Metz, France

Backgrounds

Beyond their selective properties, some antibiotics have been shown to induce the dissemination of their own resistance genes by interfering with the regulation of specific mobile genetic elements (MGEs) once at sub-inhibitory concentrations. Demonstrating such a stimulating effect remains quite difficult because there is no obvious way to identify an active antibiotic and its working concentration.

Objectives

The aim of this study was to screen and identify antibiotics for their ability to activate the transfer of known MGEs.

Methods

Inspired by J. Davies and co-workers, we developed a promoter-fusion-based screening approach where full gradients of antimicrobials can be tested instead of arbitrarily defined concentrations. To do so, bacterial laws of *lux* reporter strains are exposed on plates to a gradient of antibiotic provided by disk diffusion assays, while light emission is recorded by a CCD camera to reveal the activity of the promoters. Induction profiles are finally extracted using a homemade automated image processing software.

Conclusions

Sixty molecules, covering most classes of antibiotics, were screened for their ability to interfere with the expression of MGEs mobility functions. For MGEs such as Tn916, we could identified several modulating antibiotics for which the elements does not provide any resistant determinant, and their ability to trigger the transfer of the elements was further confirmed with standard mating assays. This observation clearly disconnects the inducer molecules from the selective advantage provided by the disseminated resistance genes, and raises the question of the risks posed by some antibiotic therapies.

FEMS7-1623
Physiology / Biochemistry / Molecular Microbiology - Part II

MORE THAN A GAME OF NUMBERS: BACTERIAL PERSISTENCE ACCELERATES THE EMERGENCE OF ANTIBIOTIC RESISTANCE BY INCREASING MUTATION RATES

J.E. Michiels[1], E.M. Windels[1], B. Van den Bergh[1], T. Wenseleers[2], M. Fauvart[3], J. Michiels[1]
[1]KU Leuven, Centre of Microbial and Plant Genetics, Leuven, Belgium
[2]KU Leuven, Laboratory of Socioecology and Social Evolution, Leuven, Belgium
[3]imec, Smart Systems and Emerging Technologies Unit, Leuven, Belgium

Backgrounds

Persisters are transient, antibiotic-tolerant variants that complicate the treatment of bacterial infections, and constitute a reservoir of viable cells in the presence of antibiotics from which resistant mutants can emerge. Moreover, stress responses implicated in persistence are also known to increase mutation rates and horizontal gene transfer. Thus, the processes of persistence and genetic adaptation could be positively coupled through common stress response mediators, fuelling the supply of resistance-conferring mutations in the persister reservoir.

Objectives

Our goal was to investigate how persistence influences the evolution of genetic resistance.

Methods

We monitored resistance by tracking (1) the development of resistant colonies on ciprofloxacin-containing agar plates, and (2) the time until the emergence of resistant mutants in liquid broth. We used natural strains from the ECOR collection and well-described high- and low persistence mutants.

Conclusions

We found a positive correlation between persister levels and the formation of resistant colonies in a collection of environmental *E. coli* strains. Additionally, we found that the size of the persister cell reservoir could only partially explain differences in resistance development. We discovered that mutation rates are pleiotropically linked with persistence and affect the likelihood that an individual persister acquires a resistance-conferring mutation. Together, our findings show that persistence shapes the evolution of genetic resistance by modulating both the number of cells surviving antibiotic treatment and the mutation rate. To conclude, we suggest that anti-persister therapies should be implemented in the ongoing battle against antibiotic resistance.

FEMS7-1095
Physiology / Biochemistry / Molecular Microbiology - Part II

H2 PRODUCTION BY ESCHERICHIA COLI BATCH CULTURES DURING FERMENTATION OF GLYCEROL AND LACTOSE AT DIFFERENT PHS

S. Mirzoyan[1], A. Poladyan[1], K. Trchounian[1], A. Trchounian[1]
[1]Yerevan State University, Biochemistry- Microbiology and Biotechnology, Yerevan, Armenia

Backgrounds

Molecular hydrogen (H_2) can be produced by microbes during utilization of cheap sources of carbon. *Escherichia coli* produces H_2 from different carbon substrates like sugars, glycerol and some organic acids.

Objectives

In this study H_2 production in batch cultures during mixed carbon sources (glycerol 10 g/l and/or lactose 1-5 g/l) fermentation at different pHs has been investigated.

Methods

H_2 generation was detected by a pair of Ti-Si and Pt redox electrodes.

Conclusions

Wild type specific growth rate on mixed carbon sources was ~1.3 fold more, compared to single carbon sources. Moreover, pH monitoring during mixed carbon fermentation showed that medium was acidified while during single lactose fermentation no pH decrease was determined.

H_2 production in wild type was detected at pH 6.5 during the utilization of lactose at various concentrations. Interestingly, no H_2 production was detected at pH 7.5 and pH 5.5. Moreover, in the mixture of glycerol and lactose at all concentrations and pHs tested H_2 generation was observed. In *hycE* (lacking large subunit of hydrogenase 3) mutant no H_2 production was detected. During growth on mixed carbon at pH 5.5, 6.5 or 7.5 in *hycE* and *hyfG* (lacking large subunit of hydrogenase 4) mutants H_2 production rate was decreased ~2.5 and ~1.5 fold, respectively, compared to wild type.

Summarizing it might be suggested that H_2 generation during the utilization of mixed carbon depends mainly on hydrogenase 3 and growth pH. These data can be applied to use different organic wastes containing glycerol and lactose and further stimulate H_2 production.

FEMS7-0227
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERIZATION OF A SEQUENCE-SPECIFIC CUTTER CONSERVED IN THE NITROSOMONAS EUROPAEA CHROMOSOME

T. Miyamoto[1], A. Yokota[2], Y. Ota[1], S. Tsuneda[1], N. Noda[2]
[1]Waseda University, Department of life science and medical bioscience, Tokyo, Japan
[2]National Institute of Advanced Industrial Science and Technology, Biomedical research institute, Tsukuba, Japan

Backgrounds

Toxin-antitoxin (TA) systems are small genetic modules that modulate microbial cell fates in stressful environments. Previously, bioinformatics analysis revealed that these TA pairs abound in some free-living microorganisms, including some chemolithotrophic prokaryotes, e.g., *Nitrosomonas europaea*. Interestingly, *N. europaea* is predicted to carry more than 50 TA pairs in its chromosome, indicating their use in coping with versatile environmental changes. However, their molecular functions are largely unknown.

Objectives

We attempted to characterize a representative TA family, the MazEF system, composed of a MazF toxin and a MazE antitoxin. Given that MazF toxins are known to be sequence-specific endoribonucleases, we isolated a putative MazF toxin encoded in the *N. europaea* chromosome and sought to identify their target sequence(s).

Methods

Unbiased RNAs were cleaved by MazF, and the position of the RNA-cleavage sites were analyzed using a specialized RNA-Seq. Subsequently, short oligonucleotides containing specific sequences inferred to be necessary for MazF cleavage were incubated with MazF, and the cleavages were monitored using a fluorometric assay.

Conclusions

We showed that a MazF homologue predicted in the *N. europaea* chromosome is a functional endoribonuclease and that it forms a canonical TA pair with its cognate MazE. Additionally, we demonstrated that the MazF toxin recognizes and cleaves a specific 3-nt motif. Our results indicate that *N. europaea* alters the translation profile and regulates its growth using this enzyme under certain stressful conditions.

FEMS7-3000
Physiology / Biochemistry / Molecular Microbiology - Part II

EXPRESSION PATTERNS OF ABC TRANSPORTER GENES IN FLUCONAZOLE-RESISTANT CANDIDA GLABRATA

M. Moazeni[1], M. Nabili[2], H. Badali[1], T. Shokohi[1], A. Abdollahi Gohar[1]
[1]Department of Medical Mycology and Parasitology- School of Medicine- Mazandaran University of M edical Sciences- Sari- Iran, Department of Medical Mycology and Parasitology, Sari, Iran
[2]Department of medical sciences- Sari Branch- Islamic Azad University- Sari- Iran, Department of medical sciences, Sari, Iran

Backgrounds

Clinical management of fungal diseases is compromised by the emergence of antifungal drug resistance in fungi, which leads to elimination of available drug classes as treatment options. An understanding of antifungal resistance at molecular level is, therefore, essential for the development of strategies to combat the resistance.

Objectives

This study presents the assessment of molecular mechanisms associated with fluconazole resistance in clinical *Candida glabrata* isolates originated from Iran.

Methods

Taking seven distinct fluconazole resistant *C. glabrata* isolates, real time PCRs were performed to evaluate the alternations in the regulation of the genes involved in drug efflux including *CgCDR1*, *CgCDR2*, *CgSNQ2,* and *CgERG11.* Gain-of-function mutations in *CgPDR1* alleles were determined by DNA sequencing.

Conclusions

Cross-resistance to fluconazole, itraconazole and voriconazole was observed in 2.5% of the isolates. In the present study, six amino acid substitutions were identified in Cgpdr1, among which W297R, T588A, and F575L were previously reported whereas D243N, H576Y, and P915R are novel. *CgCDR1* overexpression was observed in 57.1% of resistant isolates. However, *CgCDR2* was not co-expressed with *CgCDR1*. *CgSNQ2* was up-regulated in 71.4% of the cases. *CgERG11* overexpression does not seem to be associated with azole resistance, except for isolates that exhibited azole cross-resistance. The pattern of efflux pump gene upregulation was associated with gain-of-function (GOF) mutations observed in *CgPDR1*. These results showed that drug efflux mediated by adenosine-5-triphosphate (ATP)-binding cassette transporters, especially *CgSNQ2* and *CgCDR1*, is the predominant mechanism of fluconazole resistance in Iranian isolates of *C. glabrata*. Since some novel gain-of-function mutations were found here, this study also calls for research aimed at investigating other new GOF mutations to reveal the comprehensive understanding about efflux-mediated resistance to azole antifungal agents

FEMS7-3001
Physiology / Biochemistry / Molecular Microbiology - Part II

IMPROVED YEAST DELIVERY OF FLUCONAZOLE WITH A NANOSTRUCTURED LIPID CARRIER SYSTEM

M. Moazeni[1], H. Kelidari[2], R. Babaei[2], M. Saeedi[3], M. Nabili[4], A. Nokhodchi[5]
[1]Department of Medical Mycology and Parasitology- School of Medicine- Mazandaran University of Medical Sciences- Sari- Iran, Department of Medical Mycology and Parasitology, Sari, Iran
[2]Student Research Committee Center- Mazandaran University of Medical Sciences- Sari- Iran, Pharmaceutical Sciences Research Center, Sari, Iran
[3]Pharmaceutical Sciences Research Center- Mazandaran University of Medical Sciences- Sari- Iran, Pharmaceutical Sciences Research Center, Sari, Iran
[4]Department of medical sciences- Sari Branch- Islamic Azad University- Sari-Iran, Department of medical sciences, Sari, Iran
[5]Pharmaceutics Research Laboratory- School of Life Sciences- University of Sussex- Arundel Building- Brighton BN1 9QJ- UK, Pharmaceutics Research Laboratory, Brighton, United Kingdom

Backgrounds

Despite the growing trends in the number of patients at risk for invasive fungal infections, management with current antifungal agents results in complications due to changes in the epidemiology and drug susceptibility of invasive fungal infections.

Objectives

In the present research fluconazole-loaded nanostructured lipid carriers were prepared and the efficacy of the optimal formulation on a large number of Candida species was investigated.

Methods

Fluconazole-loaded nanostructured lipid carriers were prepared using probe ultrasonication technique. The morphology of the obtained nanostructured lipid carriers was characterized by transmission-electron microscopy. The minimum inhibitory concentrations (MIC) for the new formulations against strains of Candida were investigated using the Clinical and Laboratory Standards Institute document M27-A3 and M27-S4 as a guideline.

Conclusions

The fluconazole-loaded nanostructured lipid carriers presented a spherical shape with a mean diameter, zeta potential and entrapment efficiency of 126.4 ± 15.2 nm, -35.1 ± 3.0 mV, and 93.6 ± 3.5%, respectively. The drug release from fluconazole-loaded nanostructured lipid carriers exhibited burst-release behavior at the initial stage followed by sustained release over 24 hours. Using a new formulation of fluconazole led to a significant decrease in MICs for all Candida groups ($P < 0.05$). Furthermore, C. albicans isolates showed more susceptibility to fluconazole-loaded nanostructured lipid carriers than C. glabrata and C. parapsilosis ($P < 0.05$). The MIC50 drug concentration was obtained as 0.0625, 0.031 and 0.25 µg/ml for fluconazole-resistant strains of C. albicans, C. glabrata, and C. parapsilosis, respectively. In this study, we evaluated a novel delivery system which can be used as part of a strategy to improve the antifungal activity of fluconazole against various Candida strains with different susceptibilities to conventional formulations of fluconazole.

FEMS7-3113
Physiology / Biochemistry / Molecular Microbiology - Part II

ANALYSIS OF THREE PUTATIVE TRANSCRIPTIONAL REGULATORS PA1196, PA2121 AND PA2577 FROM PSEUDOMONAS AERUGINOSA PROTEOME

M. Modrzejewska[1], *A.A. Bartosik*[1]
[1]*Institute of Biochemistry and Biophysics- Polish Academy of Sciences, Department of Microbial Biochemistry, Warsaw, Poland*

Backgrounds

Pseudomonas aeruginosa, a human opportunistic pathogen, is a common cause of nosocomial infections. Its high adaptability relies on a complex network of overlapping regulatory circuits. Over 30% of its genome remains annotated as hypothetical, unknown orfs, among them many genes encoding putative transcriptional regulators (TRs). PA1196, PA2121, PA2577 belong to different families of prokaryotic-type TRs: NtrC/NifA, LysR and AsnC, respectively, with identified *in silico* HTH (helix-turn-helix) motifs, involved in DNA binding, and potential dimerization domains.

Objectives

The aim of this study was characterization of *PA1196*, *PA2121* and *PA2577*, the putative regulatory genes from *P. aeruginosa*, initially established as a part of ParA/ParB regulon.

Methods

The chromosomal deletion mutants of PAO1161 strain were constructed by allele exchange due to the homologous recombination. The *PA1196*, *PA2121*, *PA2577* genes and their promoters were cloned in appropriate vectors and used in *in vivo* experiments. The HIS-tagged TRs were overproduced and purified for *in vitro* studies.

Conclusions

PA1196, *PA2121* and *PA2577* are not indispensible for *P. aeruginosa* PAO1161 strain under tested conditions. The chromosomal mutants do not show significant differences in the growth rate, ability to metabolize different carbon sources, motility and biofilm formation in comparison to the reference strain. The purified HIS-tagged PA1196, PA2121 and PA2577 form dimers *in vitro*. The *PA2121* gene has the strongest promoter from three included in the analysis. Overproduction of PA2577, but not PA1196 or PA2121 leads to strong inhibition of *P. aeruginosa* growth.

This work was supported by the National Science Centre in Poland (grant 2015/18/E/NZ2/00675).

FEMS7-2341
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERISATION OF THE PUTATIVE TERMINATOR LOCATED UPSTREAM OF THE TN916 CONJUGATIVE MODULE

N. Mohamad Jamil[1], *P. Mullany*[1], *A.P. Roberts*[1]
[1]*UCL Eastman Dental Institute, Microbial Diseases, London, United Kingdom*

Backgrounds

Tn*916* is a clinically important conjugative transposon as it is a vector for the dissemination of antibiotic resistance among a broad range of bacteria. We identified a putative *rho*-independent terminator, 170 bp from the end of Tn*916* and upstream of the conjugation genes. The predicted termination efficiency was 31%.

Objectives

To evaluate the activity of the putative Tn*916* terminator by using *in vitro* reporter system.

Methods

To verify this experimentally, the terminator was cloned in between the *tet*(M) promoter and a *gusA* reporter gene in a pHCMCO5 shuttle vector. The termination efficiency was determined by measuring the β-glucuronidase activity of *Bacillus subtilis* containing the constructs,

Conclusions

We demonstrated the level of enzyme activity decreased by 90% when comparing the construct containing the terminator to the construct containing the promoter only, confirming termination activity. We hypothesized that the terminator is preventing the transcription of the conjugation genes when Tn*916* is integrated in the host genome. To test this, we cloned the terminator region into pHCMCO5 plus either the flanking DNA (representing the linear, integrated form) or the ligated ends of Tn*916* (representing the excised and circularized form).

The enzyme activity observed is twofold higher in the construct representing the circularized form compared to the construct representing the linear, integrated form.This data supports our hypothesis that the terminator efficiency is modulated upon excision and circularization of Tn*916*, which is the exact time when Tn*916* would require expression of its conjugation genes.

FEMS7-1420
Physiology / Biochemistry / Molecular Microbiology - Part II

REFUGEE FLOWS AND WARNING OF EMERGING RESISTANT CLONE OF MYCOBACTERIUM TUBERCULOSIS IN WEST ASIA

I. Mokrousov[1], E. Shitikov[2], Y. Skiba[3], S. Kolchenko[4], E. Chernyaeva[5], A. Vyazovaya[1]
[1]St. Petersburg Pasteur Institute, Laboratory of Molecular Epidemiology and Evolutionary Genetics, St. Petersburg, Russia
[2]Federal Research and Clinical Centre of Physical-Chemical Medicine, Laboratory of Molecular Genetics of Microorganisms, Moscow, Russia
[3]Aitkhozhin Institute of Molecular Biology and Biochemistry, Laboratory of Genome, Almaty, Kazakhstan
[4]Moscow Institute of Physics and Technology, Department of Biological and Medical Physics, Dolgoprudny, Russia
[5]St. Petersburg State University, Theodosius Dobzhansky Center for Genome Bioinformatics, St. Petersburg, Russia

Backgrounds

Mycobacterium tuberculosis has clonal population structure and some of its global lineages (e.g., Beijing, LAM) have been extensively studied. In contrast, some other remained overshadowed but the situation is changing. The underestimated NEW-1 family (prototype spoligotype SIT127), placed under the umbrella of heterogeneous Euro-American superlineage, presents a hot example. Until recently, it was endemic and prevalent in southern Iran and its historical eastwards propagation via Silk Road was hypothesized (Mokrousov, 2012). However, its multidrug resistant (MDR) subtype appears to have started a wider propagation in the region.

Objectives

We sought to correlate classical markers and whole genome sequencing (WGS) data, assess dynamic changes in phylogeography and drug resistance of NEW-1 family of *M. tuberculosis*, define its phylogeny and detect pathobiologically relevant genetic variation.

Methods

Spoligotyping, VNTR and WGS analysis was performed on DNA available in our laboratories. Published literature and databases were searched and (re)analyzed to retrieve information about SIT127 and derived strains. All data were analyzed by bioinformatics tools.

Conclusions

NEW-1 family is a part of the larger sublineage defined by RD122 deletion. In the last 15 years, NEW-1's prevalence increased significantly in North Iran, Afghanistan (proxied by data on Afghan immigrants in Iran) and Pakistan. The circulating strains are MDR-associated and this trend is also observed in farther countries, such as Bulgaria. Ongoing migration within and outside of West Asia, especially, Afghan refugees flows emphasize a risk of the wider spread of this potentially epidemic strain with arguably adverse impact on TB control in concerned countries.
Funding: Russian Science Foundation grant #14-14-00292.

FEMS7-1333
Physiology / Biochemistry / Molecular Microbiology - Part II

EFFECT OF ALLELIC DIVERSITY IN THE SIGNALING PATHWAY TORC1 ON THE NITROGEN CONSUMPTION IN SACCHAROMYCES CEREVISIAE DURING ALCOHOLIC FERMENTATION

J. Molinet[1], *F. Cubillos*[2], *C. Martínez*[1]
[1]*Universidad de Santiago de Chile, Departamento de Ciencia y Tecnología de los Alimentos, Santiago, Chile*
[2]*Universidad de Santiago de Chile,*
Centro de Estudios en Ciencia y Tecnología de Alimentos CECTA, Santiago, Chile

Backgrounds

Nitrogen is the second nutrient assimilated by yeast during alcoholic fermentation, its consumption is key for *Saccharomyces cerevisiae* to carry out this process. Deficiencies in nitrogen sources correlate with sugglish fermentation and becoming one of the main problems in the wine industry. Studies in this area have shown that different strains of *S. cerevisiae* present different levels of ammonium and amino acid consumption during fermentation. On the other hand, it is known that the TORC1 signaling pathway is responsible for detecting nutritional signals and to coordinate cell growth. Among its targets are genes related to nitrogen transport and metabolism, however, it is unknown how TORC1 can impact on the different strains generating differences in nitrogen consumption. This variability could be the result of the differences in the signaling pathway by the allelic diversity in this.

Objectives

Identify allelic variants of the TORC1 pathway involved in nitrogen consumption.

Methods

Candidate genes participating in this pathway were selected from a QTL mapping performed on a tetraparental population (SGRP-4X). The allelic variants were validated by reciprocal hemizygosity assay, which were fermented in synthetic must and determined the nitrogen content by HPLC.

Conclusions

Seven genes were selected: *GTR1, TOR2, SIT4, SAP185, EAP1, NPR1* and *SCH9*; these were validated in relation to their nitrogen consumption during fermentation process. In conclusion, allelic variants were identified in genes with different functions in the TORC1 pathway, whose allelic diversity may explain the differences in arginine, glutamic, glutamine, alanine, tryptophan and ammonium consumption.

FEMS7-1461
Physiology / Biochemistry / Molecular Microbiology - Part II

A SINGLE-MOLECULE APPROACH TO THE IN VITRO RECONSTITUTION OF THE FUNCTIONAL TYPE THREE SECRETION SYSTEM IN ENTEROPATHOGENIC E. COLI

A. Monserrat-Martínez[1], A. Ibarra-Duque[2], E. Sierecki[2], Y. Gambin[2], C.L. Marquez[2], J. Walsh[2], T. Böcking[2]
[1]Lowy Cancer Research Centre - University of New South Wales,
Single molecule science- EMBL Node- School of Medical Sciences, Sydney, Australia
[2]Lowy Cancer Research Centre - University of New South Wales,
Single molecule science- EMBL Node. School of Medical Sciences, Sydney, Australia

Backgrounds

Enteropathogenic *Escherichia coli* (EPEC) are gram-negative bacteria that can be found in the lower intestine of humans and are associated with acute gastroenteritis. EPEC relies on the type III secretion system (T3SS) or injectisome to deliver effector proteins directly into the host cells. The T3SS is distinguished by three different regions: the basal structure, the needle complex and the translocation pore.

The needle complex is responsible for bacterial attachment to the outer membrane of the host cell, after the translocation pore is inserted into the cell membrane.

Objectives

We aim to understand the way in which the needle complex is assembled and the binding steps of the formation of the translocation pore.

Methods

We use a bottom-up *in vitro* approach, where we can express the individual proteins and assess their propensity to oligomerise or fibrillate using single molecule confocal microscopy. We studied the different components of the needle and mapped their protein-protein interaction using a nanobead assay (AlphaScreen proximity assay). We then used this map to co-express protein pairs and reassemble step-by-step the full complex.

Lastly, we focus on the translocation pore and the role of EspB/EspD in attaching the tip of the needle. Here we use single-molecule Total Internal Reflection Microscopy (TIRF) to observe binding and oligomerisation on liposomes with different lipid compositions and human cell membranes. We are also studying the role of chaperones and tip attachment on the opening of the pore.

Conclusions

Eventually, we will be able to reconstruct the whole needle complex and to understand the pore formation mechanism.

FEMS7-1481
Physiology / Biochemistry / Molecular Microbiology - Part II

THE ENTEROPATHOGENIC E. COLI EFFECTORS: SWISS-ARMY KNIVES AND PATHWAY HIJACKERS

A. Monserrat-Martínez[1], A. O'Carrol[2], A. De-Andres-Murillo[2], E. Sierecki[2], Y. Gambin[2]
[1]Lowy Cancer Research Centre - University of New South Wales,
Single molecule science- EMBL Node- School of Medical Sciences, Sydney, Australia
[2]Lowy Cancer Research Centre - University of New South Wales,
Single molecule science- EMBL Node. School of Medical Sciences, Sydney, Australia

Backgrounds

Enteropathogenic *Escherichia coli* (EPEC) are gram-negative bacteria that can be found in the lower intestine of humans and are associated with acute gastroenteritis. EPEC is a highly evolved organism that only needs around 20 effectors and 20 other structural proteins to colonize a foreign environment causing disease on-set, thanks to the ability of EPEC effectors to disrupt multiple pathways in the host cell.

These effector proteins are structurally small in size (15kDa) but possess the ability to bind multiple targets associated with inflammatory response, actin reorganization, cell-to-cell junction formation and apoptosis pathways. Once in the host cells, EPEC effectors interact with some important proteins involved in innate immune system pathways, so the host cell cannot fight the invasion, eventually leading to the effective colonization of the gut.

Objectives

The aim of this project is to know what exactly the effectors are doing to immune pathways, especially the TLR, NLR and apoptotic pathways.

Methods

For that, we express the proteins of the innate immune system and systematically check whether EPEC effectors bind to them. Thanks to the cell-free expression system we use to express all the proteins, we can screen the interaction of multiple protein combinations *in vitro* using a nanobead assay (AlphaScreen proximity assay) and single molecule confocal microscopy.

Conclusions

Overall, we will establish a complete map of interactions between effectors and innate immune system proteins. The most potent effectors in terms of disruption protein-protein interactions will be studied in more detail to understand the molecular determinants of EPEC effectors versatility.

FEMS7-3127
Physiology / Biochemistry / Molecular Microbiology - Part II

SOURCE OF CARBON MAY MODULATE INDOLE-3-ACETIC ACID PRODUCTION IN ENDOPHYTIC BACTERIAL ISOLATES FROM MAIZE

A. Montanez[1], S. Vico[1], N. Rigamonti[1], A. Rodriguez[2], C. Rufo[2]
[1]Facultad de Ciencias, Montevideo, Montevideo, Uruguay
[2]Instituto Polo Tecnológico de Pando, Canelones, Pando, Uruguay

Backgrounds

Indole-3-acetic-acid (IAA) is an important phytohormone with the capacity to control plant-development in both beneficial and deleterious ways. The ability to synthesize IAA is an attribute that many bacteria including both plant growth-promoters and phytopathogens possess. Also a given bacterial species may use more than one pathway. The different IAA biosynthesis pathways using tryptophan as a precursor have been reported to be affected by environmental and genetic factors. Carbohydrate source is an important environmental factor that regulates microbial genetic expression.

Objectives

The objective of this research was to investigate the effect of different carbohydrates on the biosynthesis of IAA by endophytic bacteria isolates from maize.

Methods

Seven different carbohydrate sources were supplied to bacteria growing in minimal medium containing tryptophan. The bacteria selected for this study were: EMA68-*Pseudomona flourescens, EMA83-Rhanella* spp, EMA171-*Burkholderia cepacia*, EMA175-*Rhanella* spp, EMA176-*Rhizobium* spp**.** Filtrated supernatant samples were analyzed for IAA and biosynthetic intermediates by HPLC-MS. In order to elucidate the genes involved in IAA synthesis of our collection of IAA-producing strains, three set of primers were proposed for PCR-amplification of the genes-fragments encoding tryptophan-2-monooxygenase (*iaa*M) and IAM-hydrolase (*Iaa*H) corresponding to the indole-3-acetamide pathway and for the indole-pyruvate-pathway two set of primers were proposed for PCR-amplification of indol-pyruvate-descarboxilase (*ipd*C) gene-fragments.

Conclusions

Preliminary results showed that the regulatory mechanism controlling bacteria production of IAA biosynthesis and modulation is affected by carbohydrate source and bacteria genotype. Thus, microbial production of IAA, its impact on plant and its regulatory mechanisms represent an interesting case of plant-microbe communication and need further investigation.

FEMS7-0344
Physiology / Biochemistry / Molecular Microbiology - Part II

EXPLORING THE PROTEOSURFACEOME OF ENTEROHAEMORRHAGIC E. COLI O157:H7 DURING IN VIVO HOST-PATHOGEN INFECTION

R. Monteiro[1,2], I. Chafsey[1], C. Cordonnier[3], V. Ageorges[1], C. Chambon[4], M. Hébraud[1,4], S. Leroy[1], M. Pizza[2], V. Livrelli[3], A. Pezzicoli[2], M. Desvaux[1]
[1]Université Clermont Auvergne - INRA, UMR454 MEDiS, Clermont-Ferrand, France
[2]GlaxoSmithKline Vaccines, Antigen Identification and Molecular Biology, Siena, Italy
[3]Université Clermont Auvergne - INRA- INSERM, UMR 1071 - M2iSH, Clermont-Ferrand, France
[4]French National Institute for Agricultural Research - INRA, PFEM, Saint-Genès Champanelle, France

Backgrounds

Enterohaemorrhagic *E. coli* (EHEC) are anthropozoonotic agents that range third among food-borne pathogens respective to their incidence and dangerousness in Europe. The ability of *E. coli* to colonize different environments and to cause a wide range of disease is partly due to its capability to secrete effectors at the cell surface or in the extracellular milieu. However, their expression varies tremendously depending on the environmental conditions.

Objectives

This study aims to identify surface proteins involved in colonization by EHEC O157:H7 under intestinal conditions for future vaccination targets, following, for the first time, an analysis of the bacterial proteosurfaceome *in vivo*.

Methods

EHEC O157:H7 were cultured in BHI up to mid-exponential phase and bacterial cells were injected in the mice intestine and colon, separately. After 3 or 10 hours of incubation, EHEC cells were recovered from both intestinal sections and the bacterial proteins were tagged with Sulfo-NHS-SS biotin. Biotin-labeled surface proteins were trapped in a neutravidin column. All samples were separated by nano-HPLC and identified by high-resolution mass spectrometry.

Conclusions

The analysis of the surface-exposed proteome allowed uncovering proteins differentially expressed by EHEC during early and late state of infection and also in the different compartments of the intestinal tract. The identification of those proteins permits to understand the adaptation of EHEC, as well the proteome changes that occurs during an *in vivo* infection and leads to a successful colonization. The integration of these results with the genomic information available will help to find strategies to fight against EHEC intestinal colonization.

FEMS7-1447
Physiology / Biochemistry / Molecular Microbiology - Part II

SYNONYMOUS MUTATIONS IN THE 3-PHOSPHOGLYCERATE KINASE GENE IN SCHIZOSACCHAROMYCES POMBE ALTER PROTEIN EXPRESSION AND CELL GROWTH

S. Moreira[1], *L. Arias*[1], *O. Orellana*[1]
[1]*Universidad de Chile, Biología Molecular y Celular, Santiago, Chile*

Backgrounds

Folding of many proteins is a co-translational process, where the exit speed of the nascent peptide from the ribosome is critical. This process might be controlled by translation efficiency of the mRNA. Due to the redundancy of the genetic code, it is believed that codon bias is a relevant factor that control translation rate. So far, the rules that govern translational rate by synonymous codons and the effects on cell fitness are unknown.

Objectives

The purpose of this work was to study the effect of synonymous mutations in the gene encoding the highly expressed enzyme 3-phosphoglycerate kinase 1 (Pgk1) on protein levels and folding as well as on cell fitness of *S. pomb*e.

Methods

To study the effect of synonymous mutations, eleven segments of *pgk1* were randomly mutated for synonymous codons and each variant was introduced in the chromosome by homologous recombination. Then, we measured cell growth in minimal medium and protein aggregation.

Conclusions

We determined that some mutations in the boundaries of Pgk1 domains altered yeast growth. A concomitant alteration of protein aggregation and sensitivity to proteasome inhibitors was observed, suggesting a role of these codons in the modulation of translation and folding. These data suggest that codon usage bias is an important regulator of proteostasis.

FEMS7-0225
Physiology / Biochemistry / Molecular Microbiology - Part II

EFFECT OF THE HFQ AND CRC PROTEINS ON THE TRANSCRIPTION, PROCESSING AND STABILITY OF THE PSEUDOMONAS PUTIDA CRCZ SRNA

S. Hernández-Arranz[1], D. Sánchez-Hevia[1], R. Fernando[1], R. Moreno[1]
[1]Centro Nacional de Biotecnología, Biotecnología Microbiana, Madrid, Spain

Backgrounds

In *Pseudomonas putida*, the Hfq and Crc proteins regulate the expression of many genes in response to nutritional and environmental cues, by binding to mRNAs that bear specific target motifs and inhibiting their translation. The effect of these two proteins is antagonized by the CrcZ and CrcY small RNAs (sRNAs), the levels of which vary greatly according to growth conditions. The *crcZ* and *crcY* genes are transcribed from promoters *PcrcZ* and *PcrcY*, respectively, a process that relies on the CbrB transcriptional activator. We found that *crcZ* can also be transcribed from the promoter of the immediate upstream gene, *cbrB*, a weak constitutive promoter. The *cbrB-crcZ* transcript originated at promoter *PcbrB* was processed to render a sRNA very similar in size to the CrcZ produced from promoter *PcrcZ*.

Objectives

We analysed whether the processed sRNA, termed CrcZ*, was functional, and its possible biological role.

Methods

Using diverse approaches we determined the processing site of the *cbrB-crcZ* transcript, the amounts of CrcZ*, and its ability to antagonize the effect of Hfq and Crc.

Conclusions

The processed CrcZ* sRNA was able to antagonize Hfq/Crc since, when provided *in trans*, it relieved the deregulated Hfq/Crc-dependent hyperrepressing phenotype of a CrcZ/CrcY-null strain. CrcZ* may help attaining basal levels of CrcZ/CrcZ* that are sufficient to protect the cell from an excessive Hfq/Crc-dependent repression. In addition, we found that Crc and Hfq increase CrcZ stability, which supports the idea that these proteins can form a complex with CrcZ and protect it from degradation by RNases.

FEMS7-1615
Physiology / Biochemistry / Molecular Microbiology - Part II

TOXIN Z TRIGGERS A SURVIVAL RESPONSE TO COPE WITH STRESS BY ALTERING THE ATP, GTP, (P)PPGPP AND C-DI-AMP POOLS

M. Moreno-del Álamo[1], *M. Tabone*[1], *V. S. Lioy*[1], *J.C. Alonso*[1]
[1]*Centro Nacional de Biotecnología-CSIC, Microbial Biotechnology, Madrid, Spain*

Backgrounds

The ζ/PezT superfamily of protein toxins are found in major human pathogens and in environmental important bacteria. ζ interacts with UNAG/ATP/GTP nucleotides, and with their antitoxin protein ε_2/PezA$_2$. The toxin-antitoxin interaction leads to the formation of a biological non-toxic $\zeta\varepsilon_2\zeta$ complex that could still interact with UNAG. Transient expression of ζ triggers a reversible state of dormancy, but a small subpopulation of persisters.

Objectives

To elucidate the connection between ζ-mediated growth-arrest and the alteration on the ATP/ GTP/(p)ppGpp/c-di-AMP/UNAG pool in the presence/absence of the second stressor ampicillin (Amp).

Methods

By means of (d)NTP/NADH-linked assay we have measured the ATPase/dATPase/GTPase activities of ζ and how ε_2 affected them. We have selectively expressed ζ/ε_2 to address whether the toxin induces tolerance/persistence and if any biological noise affected our studies. To test whether the susceptible populations were death/growth-arrested, we expressed ζ for 15-900 min, and after that ε_2. We have expressed a short-living ζ variant (ζY83C) in a set of *B. subtilis* isogenic strains (Δ*relA*, Δ*gdpP*, Δ*codY*, Δ*disA*) to test how the nucleotide signalling integrates and coordinates the toxin mode of action *in vivo.*

Conclusions

We show that ζ is a (UNAG)-dependent ATPase whose activity is inhibited by stoichiometric ε_2 concentrations. ζ promotes a multi-level response, by altering the control of the signalling purine nucleotide pool, leading to growth-arrest. ζ and Amp persisters show a different sensitivity to variations in the c-di-AMP/(p)ppGpp pools. The Amp-persisters are still susceptible to toxin action, and this additive effect is still sensitive to variations in the signalling nucleotide pool.

FEMS7-0853
Physiology / Biochemistry / Molecular Microbiology - Part II

EVALUATION OF DIFFERENT DNA EXTRACTION METHODS FOR THE DETECTION OF BOTH BACTERIA (HELICOBACTER PYLORI) AND PROTOZOA (ACANTHAMOEBA CASTELLANII) IN THE SAME SAMPLE

L. Moreno-Mesonero[1], J.L. Alonso[1], I. Amorós[1], M.A. Ferrús[2], Y. Moreno[1]
[1]Instituto Universitario de Ingeniería del Agua y Medio Ambiente- IIAMA, Universitat Politècnica de València, Valencia, Spain
[2]Departamento de Biotecnología, Universitat Politècnica de València, Valencia, Spain

Backgrounds

Large sample volumes are frequently required for the analysis of waterborne pathogens, which are usually in low numbers. This fact limits the number of samples to be processed, so, a simultaneous concentration method for all types of microorganisms is used. Consequently, it is of high importance to have an optimized DNA extraction method capable of extracting high amounts of good quality DNA from all types of microorganisms present in such samples.

Objectives

In this study, ten different DNA extraction methods were evaluated for the detection of *Helicobacter pylori* and *Acanthamoeba castellanii* in the same inoculated sample to apply the best one in concentrated water samples containing bacteria and protozoa.

Methods

Ten DNA extraction methods were evaluated [(1,2): addition of PVP solution prior to FastDNA™ SPIN Kit for Soil with (1) 4 m/s 3x30s or (2) 6.5 m/s 2x60s bead-beating; (3): addition of PVP solution prior to the mammalian cells protocol of GeneJET™ Genomic DNA Purification kit; (4,5): FastDNA™ SPIN Kit for Soil with (4) 4 m/s 3x30s or (5) 6.5 m/s 2x60s bead-beating; (6,7,8): mammalian cells protocol of GeneJET™ Genomic DNA Purification kit with incubation times at 56ºC of (6) 30min, (7) 2h and (8) 24h; (9,10) UNEX buffer protocol with (9) 4 m/s 3x30s or (10) 6.5 m/s 2x60s bead-beating]. Final DNA was subjected to qPCRs of *A.castellanii* and *H. pylori,* gel electrophoresis and measure of DNA concentration and purity.

Conclusions

Overall, the best DNA extraction method was GeneJET™ Genomic DNA Purification kit with 2h of incubation time.

FEMS7-1640
Physiology / Biochemistry / Molecular Microbiology - Part II

MEMBRANE SUGAR TRANSPORTERS IN THE YEAST KLUYVEROMYCES MARXIANUS

J. Morrissey[1], J. Varela[1], N. Montini[1], M. Oreb[2], E. Boles[2]
[1]University College Cork, Microbiology, Cork, Ireland
[2]Goethe-Universität Frankfurt, Institut für Molekulare Biowissenschaften, Frankfurt, Germany

Backgrounds

Kluyveromyces marxianus is a yeast of industrial importance because of traits such as a rapid growth rate, thermotolerance and QPS/GRAS status. The yeast is used commercially for production of enzymes, bioethanol and bioflavours.

Objectives

Our overall aim is to increase the knowledge of the genetics and metabolism of this yeast to facilitate applications as a microbial cell factory. The focus in this study is sugar transporters.

Methods

We have carried out extensive comparative genomics, expression analysis and kinetic studies to understand sugar transport and metabolism. One of the most notable findings is that, compared to its relative *K. lactis,* there are many more potential sugar transporters, mainly arising from tandem gene duplications. The role of these transporters is under investigation. Not all wild strains of *K. marxianus* are equally efficient at sugar utilisation and our data indicate that, at least in part, this is due to polymorphisms in sugar transport genes. For example, we have established that although *K. marxianus* carries 4 copies of the *LAC12* gene, which encodes a lactose permease, only one of these is a functional lactose transporter. Furthermore, *K. marxianus* strains that are unable to efficiently utilise lactose carry an allelic variant of *LAC12*. This may reflect niche specialization.

Conclusions

In *K. marxianus*, there has been a major expansion of sugar transport genes and this confers this yeast with broad metabolic flexibility. Increasing knowledge will give new insights into this family of transport proteins and open up new possibilities for strain engineering for biotechnology.

FEMS7-2155
Physiology / Biochemistry / Molecular Microbiology - Part II

DEVELOPMENT OF BACTERIOCINS AS THERAPEUTIC ANTIBIOTICS

K. Mosbahi[1]
[1]*Infection Immunity & Inflammation, Bacteriology, Glasgow, United Kingdom*

Backgrounds

In the past few years there has been a worldwide rise of antibiotic resistance. New therapeutic agents are now urgently needed to treat multi-resistant and chronic bacterial infections. Amongst those threats, *Pseudomonas aeruginosa* is a Gram-negative pathogen frequently resistant to conventional antibiotics and responsible for severe hospital-acquired blood and lung infections. A promising alternative strategy is the development of bacteriocins, potent narrow spectrum protein antibiotics as novel therapeutic antibiotics.

Objectives

Our current goal is to test the efficacy of pyocins, protein antibiotics that selectively target *P. aeruginosa*, in *in vivo* disease models. Additionally we will map the uptake pathways of pyocins to enable us to rationally design pyocin combination therapies to mitigate against the development of resistance.

Methods

Our work has demonstrated pyocin efficacy in a model of acute lung infection on post-infection administration and we are currently testing these antibiotics in a chronic infection model. Mapping the uptake pathways of pyocins is currently in progress using whole geneome sequencing of isolated pyocin resistant mutants.

Conclusions

Pyocins show efficacy in an acute model of *P. aeruginosa* lung infection and testing in a chronic model is currently underway. Our current knowledge of the uptake pathways of pyocins suggests that combinations of pyocins could be designed to prevent the development of resistance. These early stage data suggest that pyocins may make good candidates to treat highly drug-resistant *P. aeruginosa* isolates.

FEMS7-1413
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERIZATION OF A ZINC ABC TRANSPORTER OF STREPTOCOCCUS AGALACTIAE

P. Moulin[1], A. Rosenau[1,2], L. Mereghetti[1,3], A. Hiron[1]
[1]UMR 1282 Université de Tours/Inra - ISP, Bactéries et Risque Materno-Foetal, Tours, France
[2]Hôpital Trousseau-CHRU de Tours, Service de Bactériologie et Hygiène Hospitalière, Tours, France
[3]Hôpital Bretonneau-CHRU de Tours, Service de Bactériologie-Virologie-Hygiène Hospitalière, Tours, France

Backgrounds

Streptoccocus agalactiae is a versatile pathogen that can infect a broad diversity of hosts ranging from human to fish. Among *S. agalactiae* virulence factors, we are particularly interested in the *lmb-sht* locus which is mainly found in human strains. In association with five others proteins we have shown that the Lmb and Sht proteins are a part of an ABC transporter involved in zinc import.

Objectives

We wanted to study the impact of zinc on bacterial metabolism and the role of the Lmb/Adc transporter in *S. agalactiae*.

Methods

Growth assays with the different mutants for genes encoding the transporter in chemically defined medium with zinc restriction showed that the Lmb/AdcA/AdcAII and the Sht/ShtII proteins are redundant as zinc suppliers. Moreover, microscopy analyzes revealed that the absence of the transporter affects the cell morphology. Finally, competition assays between the wild type and mutant strains in human biological fluids showed that the transporter is needed for the growth and survival of the bacterium. In another part we are studying a second potential role of the Sht and ShtII proteins in the resistance to complement by survival assays in whole and decomplemented human blood.

Conclusions

We characterized in *Streptococcus agalactiae* a zinc-ABC transporter and shown that it is essential for bacterial growth and morphology in zinc-restricted environments, including human biological fluids. Experiments to determine if there is a link between the two identified roles of the Sht and ShtII proteins as zinc suppliers and in the resistance to complement are ongoing.

FEMS7-1725
Physiology / Biochemistry / Molecular Microbiology - Part II

MODULATION OF HOST-CELLULAR RESPONSES BY A BACTERIAL LIGAND VIBRIO CHOLERAE PORIN OMPU

A. Mukhopadhaya[1]
[1]*Indian Institute of Science Education and Research Mohali, Department of Biological Sciences, Mohali, India*

Backgrounds

Porins represent one of the major classes of proteins present in the outer membrane of the gram-negative bacteria. Porins primarily act as channels for solute transport. However, owing to their abundance in the bacterial outer membrane, porins are capable of performing various functions modulating bacterial as well as host cell responses. OmpU, one of the major porins of gram negative bacterial pathogen *Vibrio cholerae*, helps bacteria to survive well in the gut of the human host.

Objectives

To explore whether *Vibrio cholerae* OmpU as a bacterial ligand can modulate host's innate and adaptive responses and to study the underlying mechanism.

Methods

We have done several in vitro and ex vivo studies using ELISA, western blotting, flow cytometry.

Conclusions

We have shown that OmpU is able to induce pro-inflammatory responses through TLR-mediated pathway via the recruitment of MyD88 and NF*k*B. Further, MAP kinase pathway and AP-1 transcription factors are also involved in OmpU-mediated proinflammatory responses. Further, we also observed that OmpU can induce inflammasome activation leading to the production of IL1b. Furthermore, we observed that OmpU can induce dendritic cell activation and Th1 polarization of CD4 T cells. Another interesting observation we have made that OmpU can translocate to host cell mitochondria and can induce apoptosis like programmed cell death.

FEMS7-2088
Physiology / Biochemistry / Molecular Microbiology - Part II

PROTECTIVE EFFECTS OF MELATONIN AGAINST UV IRRADIATION AND OXIDATIVE STRESS IN SACCHAROMYCES CEREVISIAE

S. Muñiz Calvo[1], R. Bisquert[2], J.M. Guillamon[2]
[1]Instituto de Agroquímica y Tecnología de los Alimentos, Biotecnología de Alimentos, Valencia, Spain
[2]Instituto de Agroquímica y Tecnología de los Alimentos CSIC, Biotecnología, Paterna, Spain

Backgrounds

Melatonin (Mel) is considered a natural potent antioxidant molecule due to its role as a free radical scavenger. Its origin is traced back to the origin of aerobic life as an early defense against oxidative stress and radiation. More complex functions have been attributed to Mel as a result of evolution in the different biological kingdoms, comprising gene expression, enzyme activity and mitochondrial homeostasis regulation processes among others.

Objectives

We tested the antioxidant and UV protective effect of Mel in *Saccharomyces cerevisiae (Sc)* since its presence has been reported during wine fermentation. As the optimal conditions for *Sc* to synthesize Mel remain unknown we developed an intracellular Mel-charging method to test its effect against stresses.

Methods

In order to assess the ability of Mel to protect *Sc* from both stresses we performed growth tests in liquid media and viability assays by colony count after a Mel treatment, followed by stress. We also performed gene expression analysis by RT-qPCR on a selection of antioxidant genes in response to the Mel treatment under oxidative stress and UV radiation

Conclusions

Our results show a significant shortening on Mel-treated cells' lag phase over control cells under stress conditions and under normal growth conditions. Viability after an H_2O_2 stress in Mel-treated cells is 26% greater than non-treated ones, while for 15 s UVC pulse (254 nm), stress amelioration reaches 28%. Gene expression analysis showed Mel significantly modulates gene expression in unstressed cells during exponential growth phase and after 30 min of both stresses.

FEMS7-0641
Physiology / Biochemistry / Molecular Microbiology - Part II

MOLECULAR DETECTION OF TREPONEMA PALLIDUM SUBSPECIES PALLIDUM FOR THE DIAGNOSIS OF CONGENITAL SYPHILIS

G. Pinilla[1], L. Campos[1], A.T. Duran[1], J. Navarrete[1], L. Munoz[1], M.Y. Cifuentes[2]
[1]Universidad Colegio Mayor de Cundinamarca, Bogota, Bogota, Colombia
[2]Universidad Nacional de Colombia, Bogotá, Bogotá, Colombia

Backgrounds

Syphilis is a disease produced by Treponema pallidum subspecies pallidum, which impacts approximately 12 million people worldwide every year. Of these, more than 2 million are pregnant women which results in congenital syphilis, the worst form of this infection.

Objectives

Detect the presence of *Treponema pallidum* subspecies *pallidum* in clinical samples in order to diagnose congenital syphilis by means of nested PCR, PCR-RT and its concordance with serological testing.

Methods

Purified DNA from a rabbit testicle and clinical samples from patients presumptively diagnosed with congenital syphilis were used.

Three target genes (*polA, 16S ADNr y TpN47*) were amplified by conventional nested PCR and PCR-RT. The results from the amplification of the *TpN47* and *polA* genes were confirmed by sequencing. The serological tests used were VDRL, RPR y TPPA.

Conclusions

By using the *TpN47* gene, the sensitivity was 52 pg for the conventional PCR, 0.52 pg for the nested PCR, and 8,4 fg for the PCR- RT . The specificity for primer TpN47 and polA was 100%; the results of the sequencing showed a 97% identity with *Treponema pallidum*. Of the samples, 70% showed concordance between the serology and the nested PCR.

The *TpN47* gene demonstrated to be the best molecular target for the identification of *Treponema pallidum*, and PCR-RT presents itself as a promising molecular diagnosis alternative for the diagnosis of congenital syphilis.

FEMS7-2406
Physiology / Biochemistry / Molecular Microbiology - Part II

THE FIRST-DEMONSTRATED SODIUM-MOTIVE CYTOCHROME-C OXIDASE OPERATES IN NATRONOPHILIC BACTERIA

M. Muntyan[1], A. Malinen[2], D. Sorokin[3], T. Ivashina[4], D. Cherepanov[1], R. Lahti[2], G. Muyzer[5], V. Skulachev[1]
[1]Belozersky Institute of Physico-Chemical Biology- Lomonosov Moscow State University, Bioenergetics, Moscow, Russia
[2]University of Turku, Biochemistry, Turku, Finland
[3]Delft University of Technology, Biotechnology, Delft, The Netherlands
[4]Skryabin Institute of Biochemistry and Physiology of Microorganisms- Russian Academy of Sciences, Molecular Biology, Pushchino, Russia
[5]Institute for Biodiversity and Ecosystem Dynamics- University of Amsterdam, Aquatic Microbiology, Amsterdam, The Netherlands

Backgrounds

The natronophilic chemolithoautotrophic bacteria *Thioalkalivibrio* inhabit alkaline (pH 10) soda lakes at saturating salt concentrations. Like all alkaliphilic microorganisms they have an inverted pH gradient across their membranes that decreases the $\Delta\mu H^+$. For many years Na^+-pumping NADH-CoQ reductase (NQR) was the only respiratory chain enzyme shown to solve this problem. *Thioalkalivibrio versutus* has no NQR and employs cytochrome oxidases (Coxs) as terminal components of its aerobic electron transport chain.

Objectives

The aim of our study was to find out how these bacteria effectively provide themselves with energy under low $\Delta\mu H^+$.

Methods

Direct Na^+ transport measurements were performed with bacterial cells and subcellular membrane vesicles loaded with $^{22}Na^+$. Sequence data were received from Genome databank and our experiments. Phylogenetic and molecular dynamic analyses were used to predict Na^+-translocating pathways.

Conclusions

The results include observation of (i) specific Na^+ dependence of Cox activity, (ii) protonophore- or valinomycin-stimulated Na^+ pumping in cells and vesicles, (iii) no H^+ pumping in them (iv) expression of the *T. versutus cbb*$_3$ in *P. denitrificans* cells with the result of Na^+ pumping in membranes of this bacterium, and (v) a Na^+-binding coordination shell in the active center of Cox.

We provide the first direct demonstration that the *T. versutus* cytochrome *cbb*$_3$ (Cox) is a primary Na^+ pump. The Na^+-pump sequence motive has been found in several bacterial strains related to *T. versutus*. Our findings are consistent with the hypothesis of using Na^+-motive energy transducers in organisms living under low ΔpH and high salinity.

Financial support: Russian Foundation for Basic Research-grant 17-04-02173.

FEMS7-0919
Physiology / Biochemistry / Molecular Microbiology - Part II

THE EFFECT OF FLHDC HOMOLOGS OF INCOMPATIBILITY GROUP A/C PLASMIDS AND SALMONELLA GENOMIC ISLAND 1 ON ACACD-RESPONSIVE PROMOTERS

G. Murányi[1], *M. Szabó*[1], *J. Kiss*[1]
[1]*NARIC, Agricultural Biotechnology Institute, Gödöllő, Hungary*

Backgrounds

The Salmonella Genomic Island 1 (SGI1) was first described in *Salmonella enterica* serovar Typhimurium DT104 in the 80's. Since then several SGI1 variants have been recognized in *Salmonella* serovars and recently in a few *Proteus* and *Acinetobacter* strains. SGI1 contains a complex In104 integron region encoding resistance for ampicillin, chloramphenicol, florphenicol, streptomycin, spectinomycin, sulphonamides and tetracycline. Furthermore, different variants encode diverse resistance patterns, which imply serious health risk for humans and livestocks. Most of the variants share common backbone. Some ORFs of the conserved backbone of SGI1 have been proved to participate in the site-specific excision and integration of the genomic island, however majority of ORFs have unknown functions. The large conjugative plasmids of Incompatibility group A/C (IncA/C) take part exclusively in the conjugal transfer of SGI1 as particular helper.

Objectives

Aims of this work are to define the essential ORFs and non-coding sequences involved in the horizontal transfer of SGI1 and to analyse the AcaCD-responsive promoters of SGI1.

Methods

Consecutive directed deletions have been generated in the conserved backbone of SGI1 by one-step gene inactivation method. Quantifying activity of activators occurred by measuring ß-gal activity. Transcription start sites were determined by primer extension analysis.

Conclusions

We found an SGI1-encoded *acaCD* homolog activator, $flhDC_{SGI1}$, which induces SGI1 excision and activates all SGI1 promoters containing predicted AcaCD binding sites. Moreover, it can complement the transfer deficiency of the *acaCD* deletion mutant IncA/C plasmid. ß-gal assays proved that overexpression of $FlhDC_{SGI1}$ has weaker effect on these promoters than AcaCD expression.

FEMS7-2226
Physiology / Biochemistry / Molecular Microbiology - Part II

BIOCHEMICAL AND GENETICAL ANALYSIS OF THE BETA-CAROTENE SYNTHESIS IN MYCOBACTERIA OF CLINICAL INTEREST

A.M. Murillo[1], B. Luz Elena[1,2], Z. Juan David[2,3], G. Verónica[1], R. Jaime[1], R. Francois[1,4]
[1]Corporación para Investigaciones Biológicas, Bacteriología y Micobacterias, Medellín, Colombia
[2]Universidad Pontificia Bolivariana, Biología de Sistemas, Medellín, Colombia
[3]Corporación para Investigaciones Biológicas, Unidad de Micología Médica y Experimental., Medellín, Colombia
[4]Equal Opportunity Life Sciences, Molecular Biology- Genetics- Cell Biology, Saint-Etienne, France

Backgrounds

Scotochromogenic mycobacteria produce pigments either in the presence or absence of light while photochromogenic ones do so only upon photoactivation. Beta-carotene is an orange-red carotenoid of the terpenoid pigment family that has been described in mycobacteria for several decades, however very little is known about its detectable levels or about the regulation of the *crt* genes encoding its biosynthesis enzymes.

Objectives

To compare the constitutive expression levels of the *crt* genes as well as the basal beta-carotene yields in 6 mycobacterial species before and after exposure to UVB light.

Methods

Clinical isolates of *Mycobacterium tuberculosis (3), M. marinum (*3*), M. avium (*3*), M. abscessus (*3*), M. bovis (*3*) and M. kansasii (*3*)* were cultivated in Middlebrook 7H9 medium until a growth to McFarland scale 1.0 was obtained. Cultures were subsequently divided into 2 identical groups, one of which was subjected to a time course exposure to UVB light. Then, bacterial pellets were divided and processed for RNA isolation and beta-carotene extraction. *Crt* genes expression levels were assessed by semi-quantitative real time RT-PCR using SYBRGreen and beta-carotene yields were measured by HPLC-DAD at 450 nm.

Conclusions

HPLC-DAD analysis showed detectable levels of beta-carotene for all species but *M. abscessus*. Exposure to UVB rays over time shows increased coordinated expression of the *crt* genes, suggesting that carotenoid pigmentation could be part of a defense mechanism triggered by mycobacteria to protect themselves against the harmful effects of UVB rays.

FEMS7-1847
Physiology / Biochemistry / Molecular Microbiology - Part II

MODULARITY OF PLASMID REPLICONS IN PSEUDOMONAS

L. Bardaji[1], M. Añorga[1], J.Á. Ruiz-Masó[2], G. del Solar[2], J. Murillo[1]
[1]Universidad Pública de Navarra, Departamento de Producción Agraria, Pamplona, Spain
[2]Centro de Investigaciones BIológicas, Molecular Biology of Gram-Positive Bacteria, Madrid, Spain

Backgrounds

Many bacterial traits of importance for humans, such as resistance to antibiotics, pathogenicity and degradation of pollutants, are carried by plasmids, facilitating their distribution among bacterial populations by lateral gene transfer. Plasmid doubling and bacterial host range depend on its replicon, usually comprising a DNA region controlling the replication rate, a replication gene and an origin of replication.

Objectives

We wanted to evaluate the organization and functionality of replicons from virulence plasmids of *Pseudomonas*.

Methods

Different replicons from the public databases were compared and aligned *in silico*. To study their functionality, we constructed appropriate deletion derivatives, mutants and chimeric replicons, and tested their ability to autonomously replicate in plasmidless strains of *P. syringae.* Incompatibility between chimeric replicons and native plasmids was assayed in wild type strains of *P. syringae*.

Conclusions

Here we propose a conceptual advance, with a paradigm shift in the genetics and biology of replicons, by showing that four replicon families from diverse species of *Pseudomonas* are organized as combinations of functionally independent control (REx-C) and replication (REx-R) modules. Modularity of replicons fosters evolutionary innovation and likely facilitates adaptation to the bacterial host and the acquisition of complexity; we also show avoidance of plasmid incompatibility by swapping REx-C modules. Notably, a REx-C module conferred replication ability to a chromosomal region from *P. syringae* containing replicon features but unable to replicate, suggesting that modularity could enable the co-option of appropriate genes into novel replicons.

FEMS7-0359
Physiology / Biochemistry / Molecular Microbiology - Part II

A NITROGEN-REGULATED SMALL RNA CONTRIBUTES TO POST-TRANSCRIPTIONAL CONTROL OF THE STRESS-ACCLIMATION PROTEIN NBLA IN CYANOBACTERIA

I. Álvarez-Escribano[1], *A. Vioque*[1], *A.M. Muro-Pastor*[1]
[1]*Instituto Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Sevilla, Spain*

Backgrounds

Photosynthetic cyanobacteria contain light-harvesting protein complexes called phycobilisomes (PBS). Several stress situations, including nutritional deficiencies or strong light exposure, lead to PBS degradation, either to protect the photosynthetic apparatus from excess light or to recycle the amino acids resulting from proteolysis. NblA is a protein adaptor that participates in degradation of PBSs. Transcription of *nblA* is induced upon nitrogen deficiency and controlled by NtcA, a CRP-family transcriptional regulator.

Objectives

We are interested in identifying NtcA-regulated small RNAs that could participate in the regulation of adaptation to nitrogen deficiency in the model heterocystous cyanobacteria *Nostoc* sp. PCC7120. In particular, we are analyzing the possible targets of NsrR1 (nitrogen stress-repressed RNA 1).

Methods

We have verified the presence of sequences encoding NsrR1 in the available genomes from heterocystous cyanobacteria and analyzed transcription of NsrR1 in response to different nitrogen regimes and transitions both in a wild-type strain and in an *ntcA* mutant. The *nblA* mRNA is computationally predicted to be regulated by NsrR1. We have verified the interaction using a GFP-based heterologous system in *E. coli*. We have analyzed the post-transcriptional regulation of *nblA* in cyanobacterial strains with altered levels of NsrR1.

Conclusions

We show that transcription of NsrR1 is regulated by NtcA, that NsrR1 interacts with the *nblA* mRNA at positions that overlap the translation initiation region, and that NsrR1 affects the accumulation of NblA mRNA and protein in *Nostoc* sp. PCC7120. Therefore, we conclude that NsrR1 contributes to the NtcA-operated control of nitrogen metabolism in cyanobacteria via *nblA*.

FEMS7-0692
Physiology / Biochemistry / Molecular Microbiology - Part II

A CO-EXPRESSION NETWORK TO DISSECT THE TRANSCRIPTOMIC CHANGES TAKING PLACE DURING HETEROCYSTS DIFFERENTIATION IN THE CYANOBACTERIUM NOSTOC SP. PCC 7120

M. Brenes-Álvarez[1], A. Vioque[1], A. Sousa-Ortega[1], J. Georg[2], W.R. Hess[2], A.M. Muro-Pastor[1]
[1]Instituto Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Sevilla, Spain
[2]University of Freiburg, Faculty of Biology, Freiburg, Germany

Backgrounds

Nostoc sp. PCC 7120 is a filamentous cyanobacterium that, in response to nitrogen deprivation, differentiates heterocysts (specialized cells devoted to fixation of atmospheric nitrogen). Because several cell types coexist at any given time in nitrogen-fixing filaments (vegetative cells, immature heterocyst and mature, nitrogen-fixing heterocysts), their global transcriptome is complex.

Objectives

We are interested in dissecting the complex transcriptome of *Nostoc* in order to identify genes (as well as antisense RNAs) differentially expressed at successive stages of the transformation of a vegetative cell into a nitrogen-fixing heterocyst. We expect those elements to be involved in development, maturation or function of this specialized cell type.

Methods

We have built a co-expression network using transcriptomic data from microarrays (corresponding to 56 samples from different strains growing under alternative nitrogen regimes). In this network, genes and antisense RNAs with similar expression patterns are grouped together. We have used the network to identify groups of genes specifically expressed in heterocysts. The differential expression of some of these genes has been verified by fluorescence confocal microscopy of strains bearing fusions of the corresponding promoters to the *gfp* gene encoding green fluorescent protein.

Conclusions

The co-expression network allows us to identify genes differentially expressed in specific cells of the filament during their transformation into heterocysts. Some of the products of these genes are well-known but others are annotated as unknown or hypothetical. Therefore, this network could help us to identify new genes (and antisense RNAs) potentially involved in the differentiation of heterocysts.

FEMS7-2992
Physiology / Biochemistry / Molecular Microbiology - Part II

MICROSATELLITE TYPING OF LARGE COLLECTION ASPERGILLUS FUMIGATUS STRAINS IN IRAN

M. Nabili[1], T. Shokohi[2], H. Badali[2], M. Moazeni[2], S. khodavaisy[3], F. Hagen[4], J.F. Meis[5]
[1]Department of Medical Sciences- Sari branch- Islamic Azad University- Sari- Iran, Sari, Iran
[2]Department of Medical Mycology and Parasitology/Invasive Fungi Research Center- School of Medic ine Mazandaran University of Medical Sciences Sari- Iran,
Department of Medical Mycology and Parasitology, Sari, Iran
[3]Department of Medical Mycology and Parasitology- Tehran University of Medical Science- Tehran- Ir an, Department of Medical Mycology and Parasitology, Sari, Iran
[4]Department of Medical Microbiology and Infectious Diseases- Canisius Wilhelmina Hospital- Nijmege n- The Netherlands, Department of Medical Microbiology and Infectious Diseases, Nijmegen, Netherlands Antilles
[5]Department of Medical Microbiology- Radboud- Nijmegen- The Netherlands,
Department of Medical Microbiology, Nijmegen, Netherlands Antilles

Backgrounds

Because of the growing incidence of *Aspergillus* infection, typing methods of *Aspergillus* species are increasingly being used, i.e., phenotypic and genotypic analysis to study the spread and population dynamics in clinical and environmental settings, at levels ranging from a single host to large-scale ecosystems.

Objectives

So, we carried out a genetic study based on the analysis of nine microsatellite loci in a large sample of isolates from different regions of Iran to explore the genetic diversity between azole resistant and susceptible environmental and clinical *A. fumigatus* strains and to compare the data with isolates from other continent.

Methods

Sixty-six clinical and environmental strains were collected from ten provinces of Iran. The collection consisted of 43 clinical isolates from a variety of specimens, in addition 23 environmental isolates collected from soil and Hospital air samples. All strains were initially identified as *A. fumigatus* based on macroscopic and microscopic characters, the ability to grow at above 45°C, and finally reconfirmed by DNA sequencing of the partial b-tubulin gene. All *A. fumigatus* strains were subjected to microsatellite typing using three separate multiplex PCRs with a panel of nine short tandem repeats (STR) to evaluate the genetic relatedness between the isolates. The genetic relationship between the *A. fumigatus* isolates was established by comparing the profiles with BioNumerics v6.6 software.

Conclusions

The Simpson's index of diversity for all nine markers combined was calculated to be less than 0.9. The STR typing of 66 *A. fumigatus* isolates revealed 38 distinct genotypes distributed among environmental and clinical isolates. We identified 12 clones including 40 different isolates representing 60% of all isolates tested, which each clone included 2–7 isolates. In concordance with previous studies, STR typing provided to be a valuable tool for studying the molecular epidemiology and genotypic diversity of clinical and environmental *A. fumigatus* isolates with excellent discriminatory power.

FEMS7-2999
Physiology / Biochemistry / Molecular Microbiology - Part II

HIGH PREVALENCE OF CLINICAL AND ENVIRONMENTAL TRIAZOLE RESISTANT ASPERGILLUS FUMIGATUS IN IRAN: IS IT A CHALLENGING ISSUE?

M. Nabili[1], T. Shokohi[2], M. Moazeni[2], S. Khodavaisy[3], H. Badali[4]
[1]Department of Medical Sciences- Sari branch- Islamic Azad University- Sari- Iran., Department of Medical Sciences, Sari, Iran
[2]Department of Medical Mycology and Parasitology/Invasive Fungi Research Center- School of Medic ine Mazandaran University of Medical Sciences Sari- Iran, Department of Medical Mycology, Sari, Iran
[3]Department of Medical Mycology and Parasitology- Kurdistan University of Medical Sciences- Sanan daj- Iran, Department of Medical Mycology and Parasitology, Tehran, Iran
[4]Department of Medical Mycology and Parasitology/Invasive Fungi Research Center- School of Medic ine Mazandaran University of Medical Sciences Sari- Iran, Department of Medical Mycology and Parasitology, Sari, Iran

Backgrounds

Triazole antifungal agents are the mainstay of aspergillosis treatment. As highlighted in numerous studies, the global increase in the prevalence of triazole resistance could hamper the management of aspergillosis

Objectives

we aimed to study the epidemiology of triazole-resistant *A. fumigatus* and the underlying *cyp51A* mutations in viable clinical and environmental isolates in Iran during 2013-2015.

Methods

In the present three-year study, 513 samples (213 clinical and 300 environmental samples) from 10 provinces of Iran were processed and screened in terms of azole resistance (4 and 1 mg l-1 of itraconazole and voriconazole, respectively), using selective plates. Overall, 150 A. fumigatus isolates (71 clinical and 79 environmental isolates) were detected. The isolates were confirmed by partial sequencing of the β-tubulin gene. Afterwards, in vitro antifungal susceptibility tests against triazole agents were performed, based on the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document. The CYP51A gene was sequenced in order to detect mutations.

Conclusions

The MIC of itraconazole against 10 (6.6 %) strains, including clinical (n=3, 4.2 %) and environmental (n=7, 8.8 %) strains, was higher than the breakpoint and epidemiological cut-off value. Based on the findings, the prevalence of azole-resistant A. fumigatus in Iran has increased remarkablyfrom 3.3 % to 6.6 % in comparison with earlier epidemiological research. Among resistant isolates, TR34/L98H mutations in the CYP51A gene were the most prevalent (n=8, 80 %), whereas other point mutations (F46Y, G54W, Y121F, G138C, M172V, F219C, M220I, D255E, T289F, G432C and G448S mutations) were not detected. Although the number of patients affected by azole-resistant A. fumigatus isolates was limited, strict supervision of clinical azole-resistant A. fumigatus isolates and persistent environmental screening of azole resistance are vital to the development of approaches for the management of azole resistance in human pathogenic fungi.

FEMS7-1643
Physiology / Biochemistry / Molecular Microbiology - Part II

A CRISPR/CAS9 SYSTEM FOR DISRUPTION OF CARB GENE IN MUCOR CIRCINELLOIDES

G. Nagy[1], C. Szebenyi[2], A. Vaz[2], E. Tóth[2], M. Homa[2], C. Vágvölgyi[2], T. Papp[1]
[1]Hungarian Academy of Sciences University of Szeged, Department of Microbiology, Szeged, Hungary
[2]University of Szeged, Department of Microbiology, Szeged, Hungary

Backgrounds

The CRISPR/Cas9 system offers a simple method for genome editing and RNA guided mutagenesis can be performed in several different organisms. The transformation strategy generally uses the crRNA and trans-activating crRNA (tracrRNA) together to guide CRISPR-associated (Cas) nuclease to cleave double-strand breaks in the targeted DNA sequence and the cell can repair this break with different repair mechanisms.

Objectives

Mucor circinelloides is a carotene producing Mucoromycotina fungus used as a model organism in studies of the carotenoid biosynthesis. Genetic manipulation of Mucoromycotina species, based on homologous recombination, is very difficult and frequently the mitotic stability of transformants is low.

Methods

In this study, CRISPR/Cas9 was used to disrupt the *carB* gene encoding phytoene dehydrogenase of *M. circinelloides*. PEG mediated protoplast transformation was used to introduce Cas9 enzyme and a synthesized *carB* gene specific gRNA with or without a deletion cassette into the fungus.

Conclusions

In the case of the usage of only the gRNA and cas9 without deletion cassette, the molecular analysis of transformant indicated a 2300 nl gap upstream from the PAM sequence by NHEJ. The colour of mutant strains was white. If we co-transformed Cas9 and gRNA with the deletion cassette the double-strand breaks of DNA was repaired by homologous recombination. After molecular analysis of these type mutants we could prove the presence of the *pyrG*, as selection marker into the coding sequence of *carB* gene.

The study was supported by the grants LP2016-8/2016 and GINOP-2.3.2-15-2016-00035.

FEMS7-2309
Physiology / Biochemistry / Molecular Microbiology - Part II

MOLECULAR CHARACTERIZATION OF BETA-LACTAM RESISTANCE AND ASSOCIATED VIRULENCE GENES IN MDR GRAM POSITIVE AND GRAM NEGATIVE CLINICAL STRAINS ISOLATED FROM ONE BIG HOSPITAL IN BUCHAREST

H. Najee[1], I. Gheorghe[2], M. Popa[3], C. Chifiriuc[3], V. Lazar[3]
[1]Bucharest university-faculity of biology, microbiology department, Bucharest, Romania
[2]Bucharest university- Faculity of Biology, Microbiology, Bucharest, Romania
[3]Bucharest university-faculity of biology, Microbiology, Bucharest, Romania

Backgrounds

The purpose of the present study was aimed to identify the main genetic elements responsible for the beta-lactams resistance in *S. aureus* and Gram negative bacilli nosocomial recently isolated strains and to correlate their resistance profiles with the virulence genes spectrum.

Objectives

To correlate the resistance profiles with the virulence genes spectrum of clinical isolated bacteria

Methods

A total number of 27 isolates belonging to *S. aureus* (n=10), *P. aeruginosa* (n=10) and *E. coli* (n=107), clinical strains recovered from different sources (respiratory tract, wound and etc…) were collected in the Lab. of the "Prof.C. C. Iliescu" Institute of Cardiovascular Diseases, Bucharest, Romania. Strains identification was performed using automatic system Vitek2 Compact and the antibiotic susceptibility testing was investigated by agar disk diffusion (CLSI, 2016). Simplex and multiplex PCR were performed on genomic DNA in order to establish: the SCCmec type in *S. aureus* isolates; the genetic support of β-lactamases in *P. aeruginosa* (bla_{VIM}, bla_{IMP}, bla_{TEM}, bla_{CTX-M},$bla_{PER)}$ and respectively, in *E.coli* (bla_{IMP}, bla_{TEM}, bla_{CTX-M} bla_{NDM} and $bla_{OXA-48)}$ strains. The following virulence genes encoding for cell-associated and soluble virulence factors were investigated: plcH, plcN, protease IV, alginate, exotoxins (ExoU,T, and S) in *P. aeruginosa*, AggR, EAggE, VT1, VT2, pap, afa,sfa in *E. coli* and bbp, ebpS, fnbB, fnbA, fib, clfA, clfB, cna, luk-PV, hlg, tst and coagulase in *S. aureus.*

Conclusions

The microbial strains recovered from hospital environment proved to exhibit multiple drug resistance and virulence determinants, suggesting their potential to persist and initiate hospital-associated infections.

FEMS7-1621
Physiology / Biochemistry / Molecular Microbiology - Part II

A MEMBRANE-TARGETING SMALL LIPOPROTEIN "KIL" INCREASED MEMBRANE PERMEABILIZATION AND BIOFILM FORMATION IN ESCHERICHIA COLI

R. Nakao[1], Y. Yoshimasu[1], S.N. Wai[2], B.E. Uhlin[2]
[1]National Institute of Infectious Diseases, Department of Bacteriology I, Tokyo, Japan
[2]Umeå University,
Department of Molecular Biology and Laboratory for Molecular Infection Medicine Sweden MIMS- Um eå Centre for Microbial Research UCMR, Umeå, Sweden

Backgrounds

Escherichia coli is one of the most prevalent microorganisms that cause catheter-associated infections in the urinary tract. Recently, we unexpectedly found that the biofilm formation of *E. coli* was enhanced by a commonly occurring plasmid gene called *kil*. The *kil* encodes small lipoprotein, however, it remains unknown the mechanism by which Kil increases the biofilm formation.

Objectives

In the present study, we addressed to understand the mechanism of Kil-dependent biofilm formation.

Methods

E. coli BW25113 strain and the derivatives were used in the present study. DNA cassetes of *kil* locus and *kil*-FLAG fusion were cloned into an arabinose-inducible expression vector pBAD33, resulting in pRN109 and pRN132, respectively. Membrane permeability and membrane potential (ΔΨ) were investigated by flow cytometry. Subcellular localization of Kil in BW25113/pRN132 was determined by a subcellular fractionation technique, followed by immunoblot using anti-FLAG antibody.

Conclusions

Supplementation with Mg^{2+} (5 mM) to the growth media dramatically inhibited membrane depolarization and increased the CFUs, suggesting its contribution to membrane stability. In the Mg^{2+}-enriched media, BW25113/pRN109 significantly increased biofilm formation as compared to BW25113/pBAD33. Induction of Kil increased membrane permeability, but not affect the ΔΨ. Most of Kil proteins translocated to the bacterial membrane but not in the cytoplasm or periplasm. We conclude that Kil inserted into the lipid bilayer enhances the biofilm formation probably due to increased membrane permeability without killing. We suggest possible roles of the Kil-encoding plasmids in spreading the *kil* locus via horizontal transfer among diverse Enterobacteriaceae, and therefore in enhancing biofilm formation.

FEMS7-0271
Physiology / Biochemistry / Molecular Microbiology - Part II

ANTIMICROBIAL SUSCEPTIBILITY AND PHYLOGENETIC ANALYSIS OF PROPIONIBACTERIUM ACNES ISOLATED FROM ACNE PATIENTS IN A JAPANESE HOSPITAL

K. Nakase[1], *N. Hayashi*[2], *N. Noguchi*[1]
[1]*Tokyo University of Pharmacy and Life Sciences, Depertment of Microbiology, Tokyo, Japan*
[2]*Toranomon Hospital, Department of Dermatology, Tokyo, Japan*

Backgrounds

The prevalence of antimicrobial-resistant *Propionibacterium acnes* strains isolated from acne patients has been increasing in Japan.

Objectives

We should understand the current resistance rate for more effective acne treatment.

Methods

We tested antimicrobial susceptibility among *P. acnes* from acne patients having visited a specialized dermatology clinic between 2013 and 2015.

Conclusions

Rates of resistance to macrolides and clindamycin were 44.3 (31/70) and 38.6% (27/70), respectively. *erm*(X), which confers high-level clindamycin resistance (minimum inhibitory concentration ≥256 μg/ml), was detected in six isolates, whereas no resistance determinants were identified in eight strains showing high-level esistance to clindamycin. Using single-locus sequence typing, the *P. acnes* isolates were classified into five clades, with all high-level clindamycin-resistant strains lacking known clindamycin resistance determinants being grouped together (in clade F). *P. acnes* isolates from patients previously treated with macrolides and clindamycin exhibited a macrolide resistance rate (55.3%) significantly higher than that of those from patients not having received these treatments (21.7%, P <0.05). Furthermore, strains of clade F, which were very rarely isolated from healthy individuals, were more frequently recovered from patients with severe acne (40.0%) than those with mild acne (23.3%).
Our data revealed an increase in macrolide-resistant *P. acnes* prevalence in Japan due to the use of antimicrobial agents for acne treatment. Moreover, we identified strains of specific phylogenetic groups frequently associated with severe acne patients.

FEMS7-1787
Physiology / Biochemistry / Molecular Microbiology - Part II

ROLE OF IRON AND FUR-FAMILY REGULATORS IN THE CONTROL OF ECTOINES SYNTHESIS GENES IN THE HALOPHILE CHROMOHALOBACTER SALEXIGENS

E. Naranjo[1], *M. Argandoña*[1], *C. Vargas*[1], *J.J. Nieto*[1]
[1]*University of Sevilla, Dpt Microbiology and Parasitology, Sevilla, Spain*

Backgrounds

Chromohalobacter salexigens is an halophilic microorganism that synthesizes ectoines as compatible solutes in response to osmotic stress, with important applications in Biomedicine. In this bacterium iron homeostasis is related with osmoadaptation, hence the synthesis of ectoines. In the genome of *C. salexigens*, two paralogs have been found, Fur1 (1) and Fur2, both involved in iron homeostasis and osmoadaptation.

Objectives

In this study we got insight into the role of both Fur regulators in the transcriptional control of genes involved in the synthesis of ectoine (*ectABC*) and hydroxyectoine (*ectD* and *ectE*), under iron limiting or iron supply conditions at different salinities.

Methods

Gene expression analysis of different mutants strains *(*Δ*fur1,* Δ*fur2 and* Δ*fur1fur2*) were performed by qPCR and compared with those of wild type strain. Besides, the intracellular content of ectoines was quantified by HPLC-MS as well as the intracellular iron content by ICP-OES.

Conclusions

Both regulators are involved in the transcriptional control of ectoines synthesis genes, albeit with different role (activator or inhibitor) depending on the environmental conditions. The existence of an additional iron-dependent but Fur-independent control mechanism was also suggested. On the other hand, the influence of iron in both the expression of ectoines synthesis genes and in its intracellular content was clearly shown. These results confirm the complex regulation of ectoine synthesis genes and the importance of iron in the osmoadaptation of this bacterium.

(1) Argandoña et al. (2010). Appl Environ Microbiol.76 (11):3575-89.

Funding: MINECO/FEDER (BIO2015-63949-R) and Junta de Andalucía (P11-CV1-7293 MO).

FEMS7-0228
Physiology / Biochemistry / Molecular Microbiology - Part II

FREQUENCY OF GROUP B STREPTOCOCCUS CARRIAGE AMONG PREGNANT WOMEN IN SARI HOSPITALS,NORTHERN IRAN

M. Nasrolahei[1], M. Sharif[2]
[1], Sari, Iran
[2]Mazandaran University of Medical Sciences, Parasitology and Mycology, Sari, Iran

Backgrounds

Vaginal colonization with group B Streptococcus (GBS) is the predominant risk factor for the development of invasive neonatal GBS diseases.

Objectives

This study was undertaken to determine the prevalence of GBS colonization in pregnant women attending the antenatal clinic of Boo-Alicina hospital in Sari,northern Iran.

Methods

This cross-sectional study was conducted during June 2015 to April 2016 on 246 pregnant women admitted at term and in preterm labor. Socio-demographic information and clinical data pregnancy and neonatal-related complications such as previous pre-term delivery, PROM (Premature rupture of membrane), neonatal sepsis and maternal infection were collected using a structured questionnaire .Two recto-vaginal swabs were obtained from study subjects and standard microbiological methods were used to isolate GBS from specimens. The presence of the atr gene which is an essential gene and has to be expressed in all cells of this species was shown using PCR method. Statistical analysis was performed using Pearson's Chi square test.Two hundred and forty six women aged between 18-55 years (mean age of 30.73) were examined for GBS colonization. Culture and PCR positivity rate of recto-vaginal swabs were 20% and 27 % respectively. No significant difference was found in GBS colonization with mother's age, previous pre-term delivery, PROM (Premature rupture of membrane), previous neonatal sepsis and maternal Urinary tract infection. Only one risk factor has been seen in two GBS colonized mothers (i.e. in one cases premature delivery and in one other case premature rupture of membranes).

Conclusions

These results are the first record of the database in Sari which show high rate of vaginal colonization of GBS in Iranian women. However, further epidemiological investigations should be done in different parts of the country and accomplishment of routine GBS screening practices is also recommended.

FEMS7-1103
Physiology / Biochemistry / Molecular Microbiology - Part II

GLYCOSIDE HYDROLASES ENCODED BY THE METHYLOFERULA STELLATA GENOME

D.G. Naumoff[1]
[1]*Winogradsky Institute of Microbiology - Moscow - Russia, Laboratory Of Wetland Microbiology, Moscow, Russia*

Backgrounds

Methyloferula stellata AR4 is an aerobic acidophilic methanotroph of the *Alphaproteobacteria* family *Beijerinckiaceae*. It is unable to grow on any multicarbon substrates, including carbohydrates. So, it is reasonable to conclude that all of its glycoside hydrolases use substrates synthesized intracellularly. The *M. stellata* draft genome sequence has been generated at the Joint Genome Institute (GenBank, ARWA00000000.1).

Objectives

Homology search of *M. stellata* genome allowed us to reveal 30 genes encoding putative glycoside hydrolases. Based on the catalytic domains, they belong to 14 different families: GH2, GH3, GH10, GH13, GH15, GH23, GH25, GH39, GH65, GH77, GH94, GH102, GH103, and PF06202. The PF06202 family is closely related to families GH63 and GH133 of glycoside hydrolases. We used each obtained glycoside hydrolase catalytic domain as a query for screening the protein database by blastp program. As a result, we found that in the vast majority of cases the closest homologue belongs to *Alphaproteobacteria*. The only exceptions are two *M. stellata* proteins representing GH39 and GH65 families of glycoside hydrolases. Eleven closest homologues of the GH39-family putative β-galactosidase (GenPept, WP_020173696.1) belong to bacteria from phyla *Actinobacteria* and *Firmicutes* (23%–31% of sequence identity). Five closest homologues of the GH65-family putative carbohydrate phosphorylase (WP_020177329.1) belong to *Betaproteobacteria* (60%–64%).

Methods

.

Conclusions

The data obtained suggest a low input of distant lateral transfer into evolution of glycoside hydrolase genes in the obligately methanotrophic bacteria. This is unusual for other bacteria and most probably is caused by the special role of their glycoside hydrolases not involved in environmental carbohydrate utilization.

FEMS7-1455
Physiology / Biochemistry / Molecular Microbiology - Part II

PET TOXIN FROM ENTEROAGGREGATIVE E. COLI CAUSES EPITHELIAL CELL DEATH BY CLEAVING PROCASPASE-3 TO ACTIVATE CASPASE-3, INDEPENDENTLY OF CALPAIN AND CASPASE ACTIVITY

F. Navarro-Garcia[1], A. Serapio-Palacios[1], M. Rios-Vargas[1]
[1]CINVESTAV, Cell Biology, Cd. de México, Mexico

Backgrounds

Pet toxin secreted by Enteroaggregative *E. coli* (EAEC) has the ability to cause cytotoxic effects on epithelial cells, which leads to cell death. However, the cell death induced by Pet or its role on the cell death induced during EAEC infection has not been elucidated. Purified Pet and Pet-producing EAEC are able to cleave fodrin, paxillin and FAK, which are also cleaved by caspases and calpains during cell death by apoptosis and necrosis, leading to cell shrinkage, membrane blebbing and cell detachment.

Objectives

The aim of this study was to understand the role of Pet in cell death induced by EAEC

Methods

Here we have characterized the cell death induced by Pet by using confocal microscopy, flow cytometry and enzymatic activies. We found that Pet toxin is involved in the induction of cell death. Pet-induced cell death is independent of calpin and caspase activities since inhibition of these two activities did not block the cell death induced by Pet. Interestingly purified Pet induced cell death mainly by apoptosis. Pet-induced apoptosis was independent of caspases because Pet is able to cleave Procaspase-3 to produce caspase-3. This caspase-3 is active after its cleavage by Pet.

Conclusions

Thus our data indicate that Pet plays a relevant role in cell death induced by EAEC; Pet cleaves directly pro-caspase-3 for producing a specie of caspase-3 active, which leads epithelial cells to cell death by apoptosis.

FEMS7-1890
Physiology / Biochemistry / Molecular Microbiology - Part II

CHARACTERIZATION OF THE VIBRIO CHOLERAE VC1636 PROTEIN: A NEW PARTNER IN DNA REPAIR MECHANISM?

V. Negro[1], *Z. Baharoglu*[1], *D. Mazel*[1]
[1]*Institut Pasteur, Genome and Genetics, Paris, France*

Backgrounds

The bacterial SOS response is a global stress response involved in DNA-damage repair. In *Escherichia coli* it is induced after DNA damage, for example after treatment with antibiotics that target DNA. The SOS response is also involved in genome plasticity and in the acquisition of resistance to antibiotics. Unexpectedly, the SOS response is induced in *Vibrio cholerae* by exposure to sub-inhibitory concentrations (sub-MIC) of different families of antibiotics that do not target DNA, such as aminoglycosides, chloramphenicol and tetracycline. The use of a genetic screen led to the isolation of mutants in which induction of the SOS response by sub-MICs of aminoglycosides is lost. One of these mutants is inactivated for the *vc1636* gene, which encodes a putative DNA/RNA helicase. We show that the overexpression of VC1636 in an *E. coli mfd* mutant, which is very sensitive to UV exposure, complements the viability of this mutant.

Objectives

The purpose of this project is to characterize the protein encoded by the *V. cholerae vc1636* gene and to understand the induction of the SOS response by antibiotics that do not target DNA in *V. cholerae.*

Methods

Towards this goal we use several bacterial genetic assays and biochemical tests *in vivo* and *in vitro* in order to study the helicase activity of this protein and the interaction with other complexes in *V. cholerae*.

Conclusions

The preliminary results show that VC1636 could play a role in the resolution of transcription and replication conflicts that can occur after a DNA damage.

FEMS7-2225
Physiology / Biochemistry / Molecular Microbiology - Part II

DECIPHERING OMPR REGULATORY IMPACT ON THE EXPRESSION OF THE KDGR REGULON GENES INVOLVED IN PECTIN-DERIVATIVES TRANSPORT IN YERSINIA ENTEROCOLITICA

M. Nieckarz[1], *A. Raczkowska*[1], *K. Brzostek*[1]
[1]*University of Warsaw- Faculty of Biology, Department of Applied Microbiology, Warszawa, Poland*

Backgrounds

The outer membrane comparative LC-MS/MS analysis revealed oligogalacturonate-specific porin KdgM2 negatively regulated by OmpR protein in enteropathogenic *Yersinia enterocolitica.* Bioinformatic analysis of *Y. enterocolitica* genome revealed clusters of genes for a variety of pectinolytic enzymes and transport systems involved in pectin-derivatives translocation into the cells. The expression of pectin degradation and catabolism genes in plant pathogen *Dickaya dadantii* is controlled by the general repressor KdgR.

Objectives

The aim of this study was to reveal the role of OmpR in the regulation of genes involved in the pectate lyases production and transport of oligogalacturonates in *Y. enterocolitica* O:9.

Methods

The expression of *kdgM1*, *kdgM2-pelP-sghX*, *pehX* and *pelW-togMNAB* in the wild-type strain, Δ*ompR*, Δ*kdgR* and Δ*ompR*Δ*kdgR* mutants was studied by applying the translational/transcriptional fusions. The OmpR-dependent activity of the *kdgR* promoter was analyzed by qPCR. To verify whether OmpR directly regulates the expression of the particular genes the band-shift assays were performed. The pectate lyase activity in *Y. enterocolitica* strains was tested using enzyme activity plate assays.

Conclusions

OmpR was found to inhibit *kdgM2*, *kdgM1* and *kdgR* genes by binding to their promoters. In contrast, the activation of *pehX* and *pelW-togMNAB* expression by OmpR appears to be indirect. Moreover, the raised level of pectate lyase PelP in the *ompR* mutant, implying a negative role for OmpR in the enzyme production. Our data revealed that OmpR plays a dual role in controlling of genes of the KdgR regulon and support the importance of OmpR as a key regulator of pectin-derivatives transport in *Y. enterocolitica.*

FEMS7-0054
Physiology / Biochemistry / Molecular Microbiology - Part II

COMPARATIVE ANALYSES OF BACTERIAL DNA FOUND IN JAPANESE RICE WINES

M. Terasaki[1], Y. Takahashi[1], H. Nishida[1]
[1]Toyama Prefectural University, Department of Biotechnology, Imizu, Japan

Backgrounds

Japanese rice wine, Sake, is a traditional Japanese alcoholic drink that is made by fermenting rice that has been polished to remove the bran. During Sake brewing, koji mold (*Aspergillus oryzae*) converts rice starch into sugars, and then Sake yeast (*Saccharomyces cerevisiae*) converts the sugars to alcohol.

Objectives

In addition to koji mold and Sake yeast, other microbes are most likely introduced during the process of Sake production. In this study, we identified and quantified bacterial DNAs found in various Sakes.

Methods

DNAs found in various Sakes were purified and concentrated. Bacterial DNAs were amplified and quantified using qPCR. In addition, massively parallel DNA sequencing was performed to clarify the origins of extracted DNAs.

Conclusions

Concentrations of bacterial DNA extracted from Sake that had been opened for 1 year was 32 times higher than those of bacterial DNA extracted from fresh Sakes. The massively parallel DNA sequence analysis showed that the old Sake included *Lactobacillus fructivorans* as the most dominant sequence, indicating that this bacterium was most likely responsible for the Sake becoming putrid. In addition, the analysis showed that, although concentrations of bacterial DNAs were similar among the fresh Sakes, variability among bacterial species was extensive. Fresh Sake includes a certain concentration of bacterial DNAs. Each Sake has a variety of bacterial DNAs, which is different from those observed in other Sakes. The variation in bacterial species may be caused by the process of Sake brewing. When bacterial species grows in Sake, the bacterial DNA can be measured as an increase.

FEMS7-3042
Physiology / Biochemistry / Molecular Microbiology - Part II

VIRUSES TAKE ADVANTAGE OF THE CAPACITY OF STING ALTERNATIVE SPLICED ISOFORMS TO ALTER THE STABILITY OF WT STING

E. Nistal-Villán[1], E. Rodríguez García[2], C. Olagüe[2], R. Ferrero[2], P. Fortes[2], J. Prieto[2], G. González-Aseguinolaza[2]
[1]Universidad San Pablo CEU-Facultad de Farmacia.,
Secciíón de Microbiología. Dep. de Ciencias Farmacéuticas y de la Salud., Boadilla del Monte, Spain
[2]Centro para la Investigación Médica Aplicada CIMA, Terapia y Expresión Génicas, Pamplona, Spain

Backgrounds

The innate immune system provides the first line of defense against pathogens in higher eukaryotes. Stimulator of interferon genes (STING), codified by the TMEM173 gene, is a critical protein involved in IFN-β induction in response to cytoplasmic DNA detection resulting from the infection by different pathogens.

Objectives

To describe the expression and function of the different alternative spliced TMEM173 mRNA species in human cells.

Methods

Over-expression and silencing effects of the different alternative splicing isoforms in activating IFN-β and antiviral programs was tested. Reporter luciferase assays, endogenous gene expression by qPCR, Western blot, bioinformatics analysis as well as quantification of viral growth were used in order to test different hypothesis.

Conclusions

Three alternative spliced STING isoforms were discovered. They fail to induce IFN-β production and act as selective pathway inhibitors of full-length (Wt) STING. Truncated isoforms alter the stability of Wt STING reducing protein half-life. The underlying mechanism of this inhibitory effect indicates a proteasome dependent degradation. Knocking-down expression of truncated isoforms increases the capability of THP1 cells to produce IFN-β in response to intracellular DNA or HSV-1 infection. Viruses like HSV-1 or VSV early after infection reduce the Wt STING/ truncated STING isoforms ratio, suggesting a manipulation of STING alternative splicing as a strategy to evade antiviral responses. Finally, *in silico* analysis reveals that the human intron-exon gene architecture of TMEM173 (splicing sites included) is preserved in other mammal species, predominantly primates, stressing the relevance of alternative splicing in regulating STING antiviral biology.

FEMS7-3230
Physiology / Biochemistry / Molecular Microbiology - Part II

PROBING THE ROLE OF CHROMATIN STRUCTURE IN THE EMERGENCE OF PATHOGENICITY IN CANDIDA GLABRATA

E. Hyland[1], C. O' Kane[2], F. Lundy[3], S. Gollogly[1]
[1]Queen's University Belfast, School of Biological Sciences, Belfast, Ireland
[2]Queen's University Belfast, School of Biological Sciences, Belfast, United Kingdom
[3]Queen's University Belfast, School of Medicine- Dentistry- and Biomedical Sciences, Belfast, Ireland

Backgrounds

The emergence of antimicrobial resistance in pathogenic fungi poses a growing threat to global public health, which is exacerbated by a limited range of antifungal therapies. Candidiasis accounts for 25% of fungal infections in the UK, and there is currently no successful treatment for this disease.

Objectives

Therefore, we aim to elucidate the molecular mechanisms behind the emergence of pathogenicity in the virulent yeast *Candida glabrata*. Using phylogenetic approaches, we have identified nineteen genes under positive selection in *C. glabrata* that regulate the post-translational modification (PTM) of histone proteins, and we hypothesize that these 19 genes are important for pathogenicity.

Methods

We are currently optimizing a two-plasmid CRISPR/Cas9 system for use in *C. glabrata* strains, in order to disrupt each of the 19 target genes, whereby, the Cas9 enzyme is maintained on one plasmid and specific guide RNA molecules are Gibson assembled into a second distinct plasmid. Once generated, these deletion strains will be assessed in clinically relevant phenotyping assays to determine the contribution of these histone PTM genes to *Candida* infections.

Conclusions

Preliminary data support our hypothesis as we have found that the deletion of either cg-*HAT1* (a histone H4 acetyltransferase) or cg-*SSP1* (a regulator of histone H3 methylation) promotes *C. glabrata*'s biofilm formation and resistance to fluconazole as determined using a crystal-violet microtitre assay and an antifungal sensitivity assay respectively. Future work involves using RNA-seq and ChIP-seq to determine the molecular basis of these phenotypic changes as well as increasing our *C. glabrata* deletion strain collection using CRISPR/Cas9.

FEMS7-1126
Physiology / Biochemistry / Molecular Microbiology - Part II

EFFECTS OF FEED SUPPLEMENTATION WITH DRY WHEY POWDER AND CALCIUM BUTYRATE ON CAMPYLOBACTER COLONIZATION AND CAECAL MICROBIAL COMMUNITY COMPOSITION IN BROILERS

M. Ocejo[1], B. Oporto[1], A. Lanzen[2], R.A. Juste[3], A. Hurtado[1]
[1]NEIKER-Tecnalia, Department of Animal Health, Derio, Spain
[2]NEIKER-Tecnalia, Department of Conservation of Natural Resources, Derio, Spain
[3]SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Villaviciosa, Spain

Backgrounds

Poultry is the principal reservoir and source for *Campylobacter* infections. Farm interventions based on chicken feed supplementation could elicit health benefits, improve production and reduce chicken colonization.

Objectives

To assess the effects of dietary supplementation of broilers with whey (prebiotic) and calcium butyrate (salt of a short-chain fatty acid) on productive traits, histological integrity, *Campylobacter* colonization and caecal microbiota during the 42-days rearing period.

Methods

One-day-old Ross-308 chickens were assigned to 4 diets (5replicates x 30chicks x 4diets): 1) control corn-soybean based diet; 2) 6% dry whey powder supplementation; 3) 0.1% coated calcium butyrate; 4) 6% whey and 0.1% calcium butyrate supplementation. At 15-days-old, *Campylobacter jejuni* was experimentally introduced into the flock. *Campylobacter* was monitored by culture and real-time PCR and, 16S rRNA high-throughput Illumina amplicons (2X250bp paired-end reads) were analysed with DADA2, QIIME and R.

Conclusions

Supplementation with 6% whey and 0.1% coated calcium butyrate improved growth and feed efficiency, had a beneficial effect on duodenal villus integrity and decreased mortality. Six days after oral challenge, *Campylobacter* was widespread in the flock, and infection remained until the end of rearing. *Campylobacter* was detected by real-time PCR in dust, air and drinkers while animals shed culturable *C. jejuni*. No differences were observed in *Campylobacter* colonization or shedding levels associated to diet suggesting that neither of these additives gave the chicks any differential degree of protection. Microbiome composition and complexity changed rapidly with age, with older chickens having higher phylogenetic diversity, but changes associated to diet were more subtle.

FEMS7-0899
Physiology / Biochemistry / Molecular Microbiology - Part II

BIOLOGICAL VALIDATION OF PUTATIVE IRON-RELATED GENES IN STAPHYLOCOCCUS EPIDERMIDIS: HOW IRON INDUCES PHISIOLOGIC AND TRANSCRIPTOMIC CHANGES ON BIOFILM CELLS

F. Oliveira[1], *N. Cerca*[1]
[1]*Centre of Biological Engineering - University of Minho, Department of Biological Engineering, Braga, Portugal*

Backgrounds

Iron has been recognized as a key nutrient for bacterial proliferation. Regarding staphylococci, most of the knowledge on iron acquisition mechanisms comes from studies on *Staphylococcus aureus*, whereas studies on *Staphylococcus epidermidis* are still in their infancy. Despite its long-known commensal nature, *S. epidermidis* has emerged as a prominent nosocomial pathogen. Its ability to grow as a biofilm has been regarded as its major pathogenic feature. Recently, we found *S. epidermidis* biofilm formation to be highly modulated by iron availability.

Objectives

To understand the mechanisms by which iron modulates *S. epidermidis* biofilm formation.

Methods

Biofilms of three *S. epidermidis* isolates were grown under iron-deficient/enriched conditions and studied for cell cultivability, matrix composition/structure (CLSM), and gene expression (qPCR).

Conclusions

The expression of several putative iron-related genes (siderophore biosynthesis, ABC transporters) was found to be modulated by iron availability, providing a biological validation of their function on iron metabolism. Physiologically, iron excess induced changes in the cultivability of suspended but not of the biofilm-associated cells. Conversely, iron deficiency induced noticeable changes in the cultivability of both suspended and biofilm-associated cells. A temporal analysis (6-24h) revealed that iron excess/deficiency: i) impaired biomass accumulation from 6h onwards, ii) induced microcolony-like arrangements, and iii) interfered with the production of extracellular matrix, indicating that iron availability plays a pivotal role from an early biofilm development stage. This study therefore paves the way for further research on the molecular mechanisms behind *S. epidermidis* iron metabolism, while placing it as an interesting target for biofilm control purposes.

FEMS7-2618
Physiology / Biochemistry / Molecular Microbiology - Part II

TRANSCRIPTIONAL REGULATORY NETWORKS INVOLVED IN PECTIN DECONSTRUCTION IN ASPERGILLUS NIDULANS

M. Orejas[1], *A. MacCabe*[1]
[1]*Instituto de Agroquímica y Tecnología de Alimentos IATA-CSIC, Departamento de Biotecnología, Paterna, Spain*

Backgrounds

The cell walls of higher plants are composed of proteins and four polymeric building blocks: the polyphenol lignin and the three polysaccharides cellulose, hemicellulose and pectin. The utilization of the complex sugar-containing constituents is a highly regulated event in both saprophytic fungi growing on dead plant material and phytopathogenic fungi when colonizing their hosts. Pectin's chemical composition comprising various monosaccharides (*e.g.* galacturonic acid and L-rhamnose) linked by a variety of bonds makes it the most structurally complex of the plant cell wall polysaccharides, and as such its efficient degradation requires the action of a range of diverse enzyme activities.

Objectives

Using *Aspergillus nidulans* as a model, we are characterizing how filamentous fungi respond to pectin and its sugar components.

Methods

A genome-wide expression study is being performed under different growth conditions with wild type and loss-of-function mutants in two activators (RhaR and PecR; the latter was identified in this work) and the wide-domain repressor CreA.

Conclusions

RNAseq analyses reveal that: (i) regulation of the pectinolytic machinery is a highly coordinated process, (ii) L-rhamnose induces enzymes appropriate for the degradation of rhamnogalacturonan I, and (iii) RhaR and PecR have key roles in the degradation/utilization of the pectic polysaccharides rhamnogalacturonan I and II, respectively. The knowledge obtained could be used to modify the expression of sugar transporter genes, rhamnose catabolic genes and/or rhamnosidase regulatory genes with a view to modulating (up or down) the production of pectin-acting enzymes.

This work is supported by DGICYT Grant AGL2015-66131-C2-2R (MINECO/FEDER)

FEMS7-1381
Physiology / Biochemistry / Molecular Microbiology - Part II

MODULAR STRUCTURE OF SRNAS AND CODON PREFERENCE OF TARGET REGIONS IN ESCHERICHIA COLI.

O. Orellana[1], F. Avalos[2], J.P. Maldonado[3], M. Tello[2]
[1]Facultad de Medicina -Universidad de Chile, Programa de Biología Celular y Molecular- ICBM, Santiago, Chile
[2]Facultad de Química y Biología- Universidad de Santiago de Chile.,
Laboratorio de Metagenómica Bacteriana- Centro de Biotecnología Acuícola, Santiago, Chile
[3]Facultad de Medicina- Universidad de Chile,
Laboratorio de Biología Molecular Bacteriana- Instituto de Ciencias Biomédicas, Santiago, Chile

Backgrounds

Small RNAs (sRNA) are a type of non-coding RNA that control bacterial translation process modifying ribosome binding, translation progression or degradation of mRNA. sRNAs may interact with one or more mRNAs mainly in the 5'UTR and/or in coding regions (CDS). However, it is unknown whether there is a conservation of the interaction regions in the target mRNAs. It is also unknown whether the interaction is associated to some specific codons

Objectives

To assess these questions we identified in *E. coli* sRNAs with multiple target mRNAs and characterized the interacting regions.

Methods

To achieve these goals bioinformatic approaches were used. Experimental data of sRNA-mRNA interactions were obtained from RNATarBAse and BSRD databases. Uncharacterized interactions were predicted using IntaRNA software. The regions of interaction in sRNA were identified mapping the density of sRNA-mRNA interactions over the sRNA sequence. The mRNA interacting regions were characterized at the codon level using CAI and tAI indexes.

Conclusions

18 sRNAs were found with 2 to 6 interacting regions. Spot42 and other sRNAs interact manly with CDS while others with the 5'UTR, suggesting a modular structure. Although there is no preference for biased codons, most sRNAs interact in regions with lower tAI compared to the rest of mRNA, suggesting a connection between sRNA regulation and translation efficiency. Experiments to validate the bioinformatic predictions are currently in progress.Our results suggest that sRNAs show a modular structure, interacting in many cases with coding regions with low codogenic preference and tAI.

Supported by: Proyecto Fondecyt 1150834 to OO

FEMS7-3091
Physiology / Biochemistry / Molecular Microbiology - Part II

STUDY OF THE GUT MICROBIOTA IN THE PROGRESSION OF SPORADIC COLORECTAL ADENOMA-CARCINOMA SEQUENCE

B.S. Orena[1], G. Mori[1], S. Gaiarsa[1], F. Armanini[2], N. Segata[2], G. DeMaio[3], C. Rengucci[3], G. Barbieri[1], A. Passardi[4], A. Casadei Gardini[4], G.L. Frassineti[4], A. Albertini[1], G.N. Ranzani[1], D. Sassera[1], D. Calistri[3], M.R. Pasca[1]
[1]University of Pavia, Biology and Biotechnology "Lazzaro Spallanzani", Pavia, Italy
[2]University of Trento, Centre for Integrative Biology CIBIO, Trento, Italy
[3]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori I.R.S.T., Biosciences Laboratory, Meldola, Italy
[4]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori IRST IRCCS, Department of Medical Oncology, Meldola, Italy

Backgrounds

Colorectal cancer (CRC) development is a multistep process by which healthy gut epithelium slowly develops into adenomas, which in turn progress into malignant carcinomas over time. This pathogenic progression is known as the adenoma–carcinoma sequence. The formation of intestinal preneoplastic lesions and CRC are associated with several risk factors such as the microbial gut dysbiosis, supporting the concept that changes in the microbiota precede the progression into adenocarcinomas.

Objectives

This study aims to identify specific bacterial associations in adenoma-carcinoma sequence.

Methods

Faecal samples from a cohort of about 100 samples coming from healthy people (18) and patients with low-risk adenomas (LRA, 19), high-risk adenomas (HRA, 21), hyperplastic polyps (HP, 14) and with adenocarcinomas (ADK, 22) were collected. DNA was extracted and purified from all samples using QIAamp DNA stool kit (Qiagen) and was used for 16S metagenomic library preparation. These libraries were sequenced using HiSeq 2500 Illumina platform and *in silico* analysis of metagenomics data was performed. Interesting results were obtained at each taxonomic level. Some data obtained at phylum level can be summarized as follows:

- There is an increase of *Proteobacteria* in the adenoma-carcinoma sequence, confirmed also at genus level;

- In HRA and ADK samples there is a decrease in *Actinobacteria*, confirmed by a decrease in *Bifidobacterium* genus;

- *Fusobacteria,* a possible CRC driver, are more abundant in HRA.

Conclusions

Taking advantage of metagenomics approach, by this multidisciplinary study we identified specific microbial associations that can be potentially used as biomarkers during neoplastic progression.

FEMS7-0557
Physiology / Biochemistry / Molecular Microbiology - Part II

EXPRESSION AND PURIFICATION OF A RECOMBINANT THIOSULFATE DEHYDROGENASE FROM AZOSPIRILLUM THIOPHILUM BV-S AND ITS STRUCTURAL CHARACTERISATION

M. Orlova[1], E. Rogozhin[2], A. Fomenkov[3], M. Grabovich[1]
[1]Voronezh State University, Department of Biochemistry and Cell Physiology, Voronezh, Russia
[2]Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry- Russian Academy of Sciences, Department of Molecular Neurobiology, Moscow, Russia
[3]New England Biolabs, Genome Biology, Massachusetts, USA

Backgrounds

The activity of thiosulfate dehydrogenase (Tsd) from *Azospirillum thiophilum* was shown previously, but its gene was not found in the genome by automatic annotation.

Objectives

Our task was to find *Tsd* gene in the genome of *A. thiophilum*, to clone it into the plasmid and to measure the properties of the appropriate protein.

Methods

The gene encoding a full-length sequence of the hypothetical protein from GenBank Database (WP_052710250) was expressed in *Escherichia coli* BL21 (DE3) cells with induction by 0.2% arabinose. The enzyme was purified by consistent hydrophobic, size-exclusion and affinity liquid chromatography procedures of medium and high pressure. Structural identification of the isolated protein was conducted by SDS gel-electrophoresis, electro transferring to PVDF membrane, N-terminal automated Edman degradation, MALDI-TOF mass spectrometry and chemical cleavage by cyanogen bromide.

Conclusions

The analysis of sequences in the NCBI database revealed a homologous gene for *tsd* (*cytc*), encoding a protein from the family of cytochromes (WP_052710250) in the genome of *A. thiophilum*. The alignment of Tsd from *A. thiophilum* with homologous proteins revealed the conserved motifs typical for previously characterized Tsd.

Tsd gene was cloned into the plasmid pBAD34 at sites for restriction endonucleases *NcoI* and *XbaI*. After purification the active protein was obtained (concentration 0.46 mg/mL, 10-20% of the total activity). Two N-terminal amino acid residues (Met and Ala) of the recombinant protein were hypothetically cleaved by bacterial amino peptidases. Identity of three peptide fragments, resulting from chemical hydrolysis and separated by reversed-phase high performance liquid chromatography, was confirmed by MALDI-TOF MS and Edman microsequencing.

FEMS7-2488
Physiology / Biochemistry / Molecular Microbiology - Part II

THE ROLE OF LMO1216 IN GROWTH AND BIOFILM FORMATION OF LISTERIA MONOCYTOGENES

R. Ostrowski[1], M. Popowska[1]
[1]University of Warsaw - Faculty of Biology, Department of Applied Microbiology, Warsaw, Poland

Backgrounds

L. monocytogenes is a gram-positive, foodborne pathogen causing listeriosis. This species is widespread in nature which is connected with a high number of surface proteins. Very important class of these proteins are murein hydrolases (autolysins), involved in several processes, including cell growth, separation and division, biofilm formation, turnover of cell wall components, protein secretion, autolytic activity and pathogenicity. Lmo1216 is one of the hypothetical autolysins of *L. monocytogenes.* It comprises two domains: FlgJ (Flagellum-specific peptidoglycan hydrolase) and SH3 (containing GW repeat).

This work was supported by National Center of Science grant nº 2013/09/B/NZ6/00710.

Objectives

Determination of the impact of Lmo1216 autolysin on growth rate and biofilm formation in *L. monocytogenes* EGD*.*

Methods

A deletion in *lmo1216* gene was obtained by cloning its fragments in tandem in pMAD plasmid. Using this construct, a mutagenesis procedure was performed according to standard protocol. Optical density was measured in 30 minutes intervals to estimate growth rate in BHI medium at 20ºC, 30ºC, 37ºC with shaking at 120 rpm. Level of biofilm formation by bacteria was assessed by staining with 1% crystal violet. In both experiments the impact of SDS, Triton X-100, EDTA was also investigated. We performed standard motility tests and cell morphology was checked by transmission electron microscopy.

Conclusions

The *lmo1216* gene takes part in growing of *L. monocytogenes* significantly decreasing the growth rate. Lack of *lmo1216* gene influences the level of biofilm formation by this bacterium. We also observed changes in morphology and motility of the mutant.

FEMS7-2981
Physiology / Biochemistry / Molecular Microbiology - Part II

PROTEIN ARGININE PHOSPHORYLATION IN STAPHYLOCOCCUS AUREUS

S. Junker[1], S. Maass[1], A. Otto[1], M. Hecker[2], D. Becher[1]
[1]Institute for Microbiology, Microbial Proteomics, Greifswald, Germany
[2]Institute for Microbiology, Microbial Physiology, Greifswald, Germany

Backgrounds

As human pathogen known to be the causative agent of a high number of nosocomial infections worldwide, *Staphylococcus aureus* is regarded a severe threat. One approach in research to understand pathogenesis and virulence on molecular level is the investigation of the phosphoproteome revealing central hubs of regulation. This has already been done for well-known o-phosphorylations and phosphorylations on cysteine, histidine and aspartate residues, and has recently been applied also to phosphorylations at arginine residues. It is likely that the latter play an essential, but mostly still unknown role in Gram positive bacteria.

Objectives

In *S. aureus* COL, the protein PtpB is assumed to be an arginine phosphatase. Hence, in the present work, we applied a gel-free method to analyze the changes in the phosphoproteome of the deletion mutant Δ*ptp*B and the wild type, focusing on analysis of arginine phosphorylations.

Methods

With the help of spectral library enhanced processing of LC-MS/MS datasets acquired from phosphoenriched samples, we are able to identify 207 arginine phosphorylations exclusively within the Δ*ptp*B mutant. The spectral library used for both enhancing the number of identifications and reproducibility of data processing comprises 84% of the theoretically predicted proteome with a total of 396 arginine phosphopeptides. A subset of the analytically challenging arginine phosphopeptides used for library building was verified by complementary analysis of chemically synthesized phosphopeptides.

Conclusions

The identification of putative targets of PtpB allows for further investigations of the physiological relevance of arginine phosphorylations and provides the basis for reliable quantification of this protein modification in *S. aureus*.

FEMS7-3059
Physiology / Biochemistry / Molecular Microbiology - Part II

BIOCHEMICAL CHARACTERIZATION OF N-TERMINALLY TRUNCATED FTSH PROTEASE OF GEOBACILLUS KAUSTOPHILUS

I. Ozdemir[1], S.S. Ramadhan[1], A. Tulek[1]
[1]Gebze Technical University, Molecular Biology and Genetics, Kocaeli, Turkey

Backgrounds

In both eukaryotic and prokaryotic cells, protein degradation is largely conducted by ATP-dependent proteases such as Lon, FtsH, ClpAP, ClpXP and HslUV. Among these proteases, FtsH is unique as a membrane-bound ATP-dependent zinc-metalloprotease that degrades integral membrane proteins as well as cytoplasmic proteins. It spans the cytoplasmic membrane twice and has a large cytoplasmic C-terminal with a putative ATP binding domain.

Objectives

We studied ATPase and proteolytic activity of soluble FtsH protease from thermophilic *Geobacillus kaustophilus* strain DSM 7263^T which lack the transmembrane helices.

Methods

The 1530 bp gene was cloned in expression vector pET14b and expressed in BL-21(DE3) host cells. The recombinant protein contained hexahistidine tag at N-terminus and was purified using nickel-NTA chromatography. The molecular weight of N-terminally truncated *G.k*-FtsH was determined by SDS-PAGE and Western Blotting as ~58 kDa. Optimum temperature for ATPase activity was observed at 55˚C and the enzyme retained 60% activity after half-hour incubation at 55-60˚C. *G.k*-FtsH protease requires both ATP and Mg^{2+} for its ATPase activity, 3.4 U/µL. The enzyme is also capable of hydrolyzing CTP and slightly GTP but not UTP and ADP. ATPase activity of *G.k*-FtsH is inhibited by 10 mM metal-chelating agents EDTA and O-phenanthroline, 65% and 48% respectively and markedly inhibited in the presence of 1 mM lactacystin and PMSF. Purified *G.k*-FtsH degraded α-casein efficiently in the presence of 4 mM ATP at up to 96-98% of the starting concentration.

Conclusions

These results showed that without N-terminal transmembrane domain *G.k*-FtsH successful expressed and showed efficient ATPase activity and protease activity.

FEMS7-0999
Physiology / Biochemistry / Molecular Microbiology - Part II

PHYSIOLOGICAL ROLE OF THE LOW-MOLECULAR-MASS PENICILLIN-BINDING PROTEIN DACC IN ACINETOBACTER BAUMANNII

S. Pal[1], D. Kar[1], G. Kumar[1], A. Kumar[1], A.S. Ghosh[1]
[1]Indian Institute of Technology Kharagpur, Biotechnology, Kharagpur, India

Backgrounds

Multi-drug resistant *Acinetobacter baumannii* has emerged as an important nosocomial pathogen. As some low-molecular-mass penicillin-binding proteins are known to possess intrinsic β-lactam resistance properties, it would be interesting to unravel the physiological role of DD-Carboxypeptidases in *Acinetobacter baumannii*.

Objectives

In this study we aimed to characterize the physiological role of DacC of *A. baumannii* in β-lactam resistance, biofilm formation and cell shape maintenance.

Methods

The *dacC* gene was deleted from the genome of *A. baumannii* ATCC19606 and the membrane-bound form of the protein was cloned in pBAD18-Cam for expression in *Escherichia coli* and pABBR-MCS for *A. baumannii*). Biofilm formation was assessed by crystal violet staining. The minimum inhibitory concentrations of the β-lactams were determined using broth micro-dilution method. Cell morphology was analysed by phase contrast microscopy. To confirm the biochemical reason behind the physiological behaviours, the soluble form of the protein was created, expressed and purified. Steady state kinetic studies were done with the β-lactams and the peptide substrates.

Conclusions

The deletion of *dacC* resulted in reduced biofilm formation and also led to an abnormal cell shape. It also made the cells sensitive to some β-lactam antibiotics. Ectopic complimentation restored the lost phenotypes. The soluble protein exhibited DD-Carboxypeptidase activity against peptidoglycan-mimetic DD-Carboxypeptidase substrates as well as showed efficient β-lactam hydrolysis. Our hypothesis is that *A. baumannii* DacC is a dual enzyme, possessing both DD-Carboxypeptidase and β-lactamase activities, that is involved in biofilm formation and helps in maintaining cell shape.

FEMS7-1425
Physiology / Biochemistry / Molecular Microbiology - Part II

LIFE WITHOUT CROSSING-OVER: GENOME EVOLUTION AND MECHANISMS OF MEIOSIS IN THE NEW MODEL YEAST SACCHAROMYCODES LUDWIGII

I.A. Papaioannou[1], N. Delhomme[2], F.A. Peltier[1], A. Bardhan[1], W. Wu[3], L. Steinmetz[3], M. Knop[1]
[1]University of Heidelberg,
Zentrum für Molekulare Biologie der Universität Heidelberg - ZMBH - ZMBH-DKFZ Alliance, Heidelberg, Germany
[2]University of Umeå, Department of Plant Physiology - Umeå Plant Science Center, Umeå, Sweden
[3]European Molecular Biology Laboratory - EMBL, Genome Biology Unit, Heidelberg, Germany

Backgrounds

The paradox of sex, i.e., its ubiquity despite its biological costs, is one of the most persistent conundrums in biology. Most relevant hypotheses focus on the roles of meiotic recombination in handling mutations, e.g., purging of the deleterious load and generation of new combinations of beneficial alleles. However, the impact of recombination on genome evolution is still poorly understood.

Objectives

To analyze the diploid yeast *Saccharomycodes ludwigii* as an ideal candidate for the study of genome evolution and the mechanisms of meiosis in the absence of crossing-over. This species experiences a full sexual cycle but generally fails to recombine its genome during meiosis.

Methods

The genome of *S. ludwigii* was sequenced, assembled and annotated. Repetitive content in DNA and protein sequences was compared with 23 other sequenced yeast species. A mutation accumulation experiment and a variant segregation analysis in full sequenced tetrads of a hybrid diploid strain were performed. Deletion/tagging strains of important meiotic genes were constructed and analyzed for putative functionality in meiosis.

Conclusions

Saccharomycodes ludwigii was established as a particularly interesting organism for the study of genome evolution. We are proposing an evolutionary model entailing the shaping of a remarkably A/T-rich genome as a consequence of the absence of crossover-associated gene conversion and the biased nature of mutations; the accumulation of repetitive DNA elements including particular codon expansions in genes; and the significant predicted expansion of prion-like proteins as a conceivably efficient way to ensure adequate levels of adaptation potential despite the absence of meiotic recombination.

FEMS7-1431
Physiology / Biochemistry / Molecular Microbiology - Part II

SEX IS NOT THE ONLY WAY: NEW INSIGHTS INTO PARASEX IN THE PLANT-PATHOGENIC FUNGUS VERTICILLIUM DAHLIAE

I.A. Papaioannou[1], V. Vangalis[2], M.A. Typas[2]
[1]University of Heidelberg, Zentrum für Molekulare Biologie der Universität Heidelberg - ZMBH, Heidelberg, Germany
[2]National and Kapodistrian University of Athens,
Faculty of Biology - Department of Genetics & Biotechnology, Athens, Greece

Backgrounds

Parasex, as an alternative to sex permitting genetic exchange between individuals, has been established and exploited for genetic analysis in several asexual fungi. However, the traditional assumption that vegetative incompatibility barriers generally hamper parasexual exchanges in natural populations, has discouraged the detailed characterization of the process in recent years, thus hindering a clear understanding of its significance in fungal evolution and population dynamics.

Objectives

(i) to elucidate poorly understood steps of the parasexual cycle in the important plant-pathogenic asexual fungus *Verticillium dahliae*, and (ii) to examine the potential impact of vegetative incompatibility on the parasexual process in this species.

Methods

Widely used tester strains of *V. dahliae* vegetative compatibility groups (VCGs) were labeled with complementary combinations of auxotrophic, drug-resistant and fluorescent markers, and used for heterokaryon synthesis tests. Hyphal fusion (anastomosis), nuclear migration, diploid formation and haploidization were observed microscopically, analyzed in terms of temporal patterns by time-lapse microscopy, monitored genetically with conidial analyses (FACS), and characterized with genomic approaches.

Conclusions

All phases of the parasexual cycle of *V. dahliae* were characterized in detail with a combination of different experimental approaches. Heterokaryon synthesis was found to be very frequent under proper, low-nutrient conditions, not only between “compatible”, but also between supposedly “incompatible” strains. Diploid formation and segregation into novel recombinant mycelia were also recorded in both “compatible” and “incompatible” combinations. These results imply that the parasexual cycle may be more important in nature than previously anticipated, and lay the foundation for detailed investigations of its evolutionary significance.

FEMS7-1870
Physiology / Biochemistry / Molecular Microbiology - Part II

SCREENING OF FOOD LACTIC ACID BACTERIA LACCASES

I. Pardo[1], M. Abril[1], R. Sendra[2], S. Ferrer[1], S. Callejón[1]
[1]ERI BioTecMed, ENOLAB, Burjassot, Spain
[2]Facultat de Biologia, Departament de Bioquímica, Burjassot, Spain

Backgrounds

Laccases (EC 1.10.3.2) are copper-containing oxidases found in many plants, fungi, animals and microorganisms. Laccases catalyse the oxidation of a variety of substituted phenols and many other compounds without any other requirement than oxygen from the air. As a result, these enzymes are promising green biocatalysts for several industrial sectors such as textile, food, wood and pulp, bioremediation, organic synthesis or electrocatalysis.

Objectives

The search of laccase enzymes in food lactic acid bacteria (LAB). The most representative LAB species from food or beverages - such as cheese, meat, olives or wine - were analysed using a microplate method specifically developed.

Methods

Laccase activity was measured from cell-free extracts using ABTS as substrate. Cells from a MRS culture were mechanically disrupted and the resulting extract clarified by centrifugation and submitted to ammonium sulphate fractionation. Then, the 90% saturation-precipitate was dissolved and saved as crude cell-free extract. Aliquots of extracts were incubated in sodium acetate buffer, pH 4.0, containing $CuCl_2$ 0.1 mM, for 10 minutes and after centrifugation, the supernatant transferred to a multiwell microplate. ABTS was added at 5 mM and its oxidation was determined by absorbance increase at 420 nm ($\varepsilon 420$=36,000 M^{-1} cm^{-1}). The initial rates were deduced from the slope of plots of A_{420} versus time, and specific activity was calculated by dividing the slope between the total protein concentration.

Conclusions

A new multiwell microplate method has been developed to detect and quantify laccase activity in LAB.

We obtained laccase positive strains from several LAB species.

FEMS7-2296
Physiology / Biochemistry / Molecular Microbiology - Part II

FUNCTIONAL CHARACTERIZATION OF ENVIRONMENTAL LACTATE AND SIALIC ACID UTILIZATION DURING NTHI IN VITRO INFECTION OF HUMAN EPITHELIA

C. Parillo[1,2], B. Baddal[3], C. Baldari[1], S. Rossi Paccani[2], A. Pezzicoli[2]
[1]Università degli studi di Siena, Biochimica e Biologia Molecolare, Siena, Italy
[2]Glaxo Smith Kline Vaccines, Antigen Identification & Molecular Biology, Siena, Italy
[3]Harvard Medical School,
Division of Infectious Diseases- Boston Children's Hospital and Department of Pediatrics, Boston, USA

Backgrounds

Non-typeable Haemophilus influenzae (NTHi) is a small acapsulate gram-negative coccobacillus and a human-restricted nasopharynx commensal. Nevertheless, it is the main cause of chronic obstructive pulmonary disease (COPD) exacerbation. A previous dual RNA-seq study showed the upregulation of *L-lactate permease* gene, suggesting a role for environmental lactate during the infection. Furthermore a strong downregulation of a *tripartite ATP-independent periplasmic transporter* was reported, while a *putative sialic acid transporter* gene was found to be upregulated during the infection.

Objectives

In order to clarify the impact of these transport systems on lactate and N-acetyl neuraminic acid (NANA) uptake, we produced the corresponding *knock out* strains and we are currently investigating their role in human bronchial epithelia infection.

Methods

Bacterial strains were grown in a chemically defined medium in order to control the lactate and NANA content. Then human bronchial epithelia were infected at different timepoints to analyse bacterial fitness and resistance to complement mediated killing.

Conclusions

Preliminary data obtained by comparing *wild type* strain with *lactate permease knock out* after 72 hours infection of a bronchial epithelium, showed an increased amount of mutant bacterial cells. We are currently investigating the mechanisms causing this apparent discrepancy. On the other hand the serum resistance of *putative sialic acid transporter knock out* is comparable to the *wild type* after the colonization of human bronchial epithelia. In conclusion our work is aimed to better characterize NTHi infection factors involved in the colonization of the human bronchial epithelium.

FEMS7-1938
Physiology / Biochemistry / Molecular Microbiology - Part II

DUAL-SPECIFICITY LAMMER KINASE REGULATES THE G1/S CELL CYCLE PROGRESSION AT TRANSCRIPTIONAL AND POST-TRANSLATIONAL LEVEL IN FISSION YEAST

S.J. Kwon[1], S. Shin[1], H.M. Park[1]
[1]Chungnam National University, Microbiology & Molecular Biology, Daejeon, Republic of Korea

Backgrounds

A number of studies have reported that the dual-specificity LAMMER kinases regulate multiple cellular processes in higher eukaryotes. While LAMMER kinase-dependent phosphorylation of Thr110 residue on Rum1, a cyclin-dependent kinase inhibitor (CKI), is crucial for G1/S cell cycle progression (Yu et al, 2013), much remains to be determined in terms of the mode of Lkh1 action in regulating cell cycle of the fission yeast *Schizosaccharomyces pombe*.

Objectives

Testing the effect of Thr110 phosphorylation of Cdc2-inhibitor Rum1 on CKI activity and affinity to CDK

Identification and characterization of the additional LAMMER kinase target(s)

Methods

CKI activity of Rum1 proteins was measured by using Cdc2-cyclin and Histone H1 assay system.

Affinity of Rum1 proteins to cyclin-dependent kinase Cdc2 was measured by pull-down assay.

Microarray analysis was performed to identify the LAMMER kinase target genes.

Kinase assay and PMF were applied to identify the LAMMER kinase target site(s) on the MBF components.

Conclusions

Kinas assay with phospho-mimic and phospho-defective Rum1 indicated that LAMMER kinase-dependent phosphorylation of Thr110 was pivotal for its CKI activity. No significant difference in affinity to Cdc2, however, was observed among Rum1 proteins. Microarray analysis with the *lkh1*$^{+}$ deletion mutant revealed four cell cycle genes up-regulated, of which expression are known to be modulated by the G1/S specific MBF (*Mlu*1 box binding factor). Modulation of MBF activity by LAMMER kinase-dependent phosphorylation of a negative regulator for MBF was also indicated. Results presented here indicate a potential dual action of LAMMER kinase in G1/S progression of *S. pombe* cell cycle: activation of Cdc2-inhibitor Rum1 and of the MBF-repressor.

FEMS7-0516
Physiology / Biochemistry / Molecular Microbiology - Part II

TLR2 INDUCTION OF NF-KB SIGNALLING PATHWAY WITH LACTOBACILLUS FERMENTUM L930BB AND BIFIDOBACTERIUM ANIMALIS SUBSP. ANIMALIS IM386 AND THEIR PROPHYLACTIC EFFECT IN DSS-INDUCED COLITIS

D. Paveljšek[1], K. Ivičak-Kocjan[2], R. Jerala[2], B. Bogovič Matijašić[1], I. Rogelj[1]
[1]Biotechnical Faculty- University of Ljubljana, Department of Animal Science, Domžale, Slovenia
[2]National Institute of Chemistry, Department of Synthetic Biology and Immunology, Ljubljana, Slovenia

Backgrounds

Intestinal epithelial cells express several pattern recognition receptors (PRR), including Toll-like receptors (TLR), which initiate signalling cascades that lead to activation of NF-κB and other pro- and anti-inflammatory pathways. Commensal and probiotic bacteria can regulate the level of NF-κB activity at the intestinal epithelial border and affect the mucosal immune balance.

Objectives

The current study was designed to characterise TLR2 signalling in human cell lines, triggered by isolates from bioptic samples of human colonic mucosa, *Lactobacillus fermentum* L930BB (L930BB) and *Bifidobacterium animalis* subsp. *animalis* IM386 (IM386), and to evaluate their possible beneficial effects in dextran sulphate sodium (DSS) – induced colitis in mice.

Methods

Dual luciferase NF-κB inducible reporter assay was conducted on HEK293 cells that were transiently transfected with TLR1/2, TLR2/6 and different ratios of TLR10. After transfection, cells were stimulated with different concentrations of live and heat killed L930BB, IM386 and also their conditioned media. Female C57BL/6JOlaHsd mice, pre-treated with live L930BB and IM386, were exposed to DSS for 5 days. For histological assessments the colon was cut longitudinally and the sections were stained.

Conclusions

TLR10 is the only PRR within the TLR family that is able to dampen TLR2 signalling through NF-κB and suppress immune response. We discovered that strains L930BB and IM386 beside TLR1/2 and TLR2/6 also induce signalling through TLR2/10 and reduce the NF-κB activation. Histological assessment revealed that pre-treatment with probiotic mixture results in decreased length of affected colon, reduced infiltration of neutrophils in mucosa and decreased depth of inflammation of the colon wall.

FEMS7-2534
Physiology / Biochemistry / Molecular Microbiology - Part II

STRUCTURAL AND FUNCTIONAL ANALYSIS OF ESSENTIAL EXTRACHROMOSOMAL REPLICONS (CHROMIDS) OF PARACOCCUS SPP. (ALPHAPROTEOBACTERIA)

A. Pawłot[1], J. Czarnecki[1], R. Lasek[1], M. Szuplewska[1], E. Prochwicz[1], M. Puzyna[1], A. Brozio[1], K. Kotecka[1], P. Dymek[1], P. Decewicz[1], Ł. Dziewit[1], D. Bartosik[1]
[1]Institute of Microbiology, Department of Bacterial Genetics, Warsaw, Poland

Backgrounds

Chromids are recently distinguished group of autonomous bacterial replicons, sharing some characteristics of both chromosomes and plasmids. They comprise genetic information of adaptive value as well as housekeeping genes, which make them essential for their hosts. The prevalence of chromids in bacteria and their conserved character within certain taxonomic groups suggests an important role for these replicons in the evolution of bacteria.

Objectives

The aim of the study is to identify chromids in bacteria of the genus *Paracoccus* (*Alphaproteobacteria*) and to analyze their variability and evolution.

Methods

Genomic sequencing of 13 strains of *Paracoccus* spp. was performed applying Illumina technology. Identification of essential replicons was performed using a target oriented replicon curing strategy, based on the replicon incompatibility phenomenon. Bioinformatic analyses included sequence annotation, comparative and phylogenetic analyses. Standard molecular procedures were employed for genetic characterization of the replication (REP) modules of chromids.

Conclusions

A characteristic feature of the identified *Paracoccus* spp. chromids was the presence of *dnaA*-like replication systems (REP), which are common within the large plasmids of bacteria of the *Roseobacter* clade. Two evolutionary lineages of dnaA-like REPs, which correlate with the genetic content of these replicons were distinguished. Interestingly, we found that several chromids harbored by a group of phylogenetically related *Paracoccus* species encode the DnaA protein, which is crucial for the chromosome replication initiation. Such chromid-dependent maintenance of bacterial chromosomes was not previously observed in any other bacteria.

FEMS7-2778
Physiology / Biochemistry / Molecular Microbiology - Part II

TOWARD THE IN VITRO PRODUCTION OF MAGNETOSOME-LIKE MAGNETITES THROUGH THE COMBINED EFFECT OF THE MAGNETOSOME-ASSOCIATED PROTEINS MAMC AND MMS6 FROM MAGNETOCOCCUS MARINUS MC-1

A. Peigneux[1], Y. Jabalera[1], R. Zarivach[2], M.A. Fernandez-Vivas[1], C. Jimenez-Lopez[1]
[1]Facultad de Ciencias. Universidad de Granada., Departamento de Microbiología, Granada, Spain
[2]Ben-Gurion University of the Negev, Department of Life Sciences, Beer Sheva, Israel

Backgrounds

Magnetite nanoparticles (MNPs) are interesting in biotechnology as they can be manipulated by an external magnetic field and can be functionalized with different molecules. Magnetotactic bacteria produce magnetosomes, which are the ideal single magnetic domain particle (SDP). Scaling-up magnetosome production is still challenging due to difficulties in magnetotactic bacteria growth, so protein-mediated magnetite crystals synthesis is being explored. Several magnetosome-associated proteins have already been studied, but their effect in the *in vitro* magnetite formation has been assayed by only using one at the time. This situation is probably far from what occurs in the magnetosome, in which a number of magnetosome-associated proteins are involved in the biomineralization process.

Objectives

In vitro magnetite precipitation experiments were performed in the presence of recombinant Mms6 or/and MamC from *Magnetococcus marinus* MC-1 to check the individual and combined effect of the proteins in the *in vitro* magnetite formation.

Methods

MamC and Mms6 proteins were expressed as recombinant proteins in *Escherichia coli* TOP10 strain. After their purification, *in vitro* magnetite precipitation experiments were carried out in the presence of each one and in mixtures at different concentrations.

Conclusions

Compared to magnetite crystals produced in the absence of any protein and under identical conditions, MamC controls the size of magnetite crystals and produces SDP (most of them > 30 nm) while Mms6 produces better faceted crystals. The combined effect of those proteins resulted in crystals both large in size (30 ± 10 nm) and with uniform 2D morphologies that were not obtained by using only one of the proteins.

FEMS7-0672
Physiology / Biochemistry / Molecular Microbiology - Part II

GENOME-WIDE IDENTIFICATION AND DNA-BINDING ARCHITECTURE OF THE ORPHAN RESPONSE REGULATOR HP1043 OF HELICOBACTER PYLORI

S. Pelliciari[1], E. Pinatel[2], C. Peano[2], V. Scarlato[1], D. Roncarati[1]
[1]University of Bologna, Department of Pharmacy and Biotechnology FaBit, Bologna, Italy
[2]National Research Council, Institute of Biomedical Technologies, Milan, Italy

Backgrounds

In *Helicobacter pylori*, the *hp1043* gene is one of the transcriptional regulator essential for cell viability. As such, this gene could not be deleted, supporting the hypothesis that HP1043 could be involved in the regulation of crucial cellular processes. The impossibility of generating a knock-out mutant for *hp1043* gene, or modulate the amount of HP1043 protein in the cell, has hampered the detailed characterization of its regulatory function.

Objectives

Discover all promoters bound *in vivo* by HP1043 and understand it's biological function.

Methods

Using Chromatin Immunoprecipitation-sequencing (ChIP-seq), we were able to identify genome-wide at least 37 new HP1043 binding sites. Moreover, *in vitro* DNaseI protection assays (footprints) enabled mapping of the HP1043 binding sites on a subset of the new targets, revealing the presence of a conserved nucleotide sequence motif consisting of a direct repeat. Furthermore, hydroxyl-radical probing allowed to refine the positions of HP1043 binding, suggesting that the proposed consensus motif is recognized by HP1043.

Conclusions

A significant fraction of newly identified binding sites overlaps promoter regions of genes involved in translation. Accordingly, arrest of protein translation determined induction of almost all HP1043 target genes. These observations prompted us to propose HP1043 as key regulator in *H. pylori*, likely involved in sensing and in coordinating the response to environmental stress inducing an arrest of protein synthesis. Experiments aimed to elucidate the role of each base of the consensus motif in the protein-DNA binding are in progress as well as experiments aimed to modulate its amount and/or activity *in vivo* in *H. pylori*.

FEMS7-2146
Physiology / Biochemistry / Molecular Microbiology - Part II

COMPARATIVE ANALYSIS OF GROWTH, DEVELOPMENT AND OTC PRODUCTION OF VARIOUS S. RIMOSUS PRODUCERS

T. Paradžik[1], E. Šarić[1], G. Quinn[1], M. Roje[2], M. Jurin[3], I. Hunter[4], P. Herron[4], D. Vujaklija[1]
[1]Ruđer Bošković Institute, Department for molecular biology, Zagreb, Croatia
[2]Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Zagreb, Croatia
[3]Ruđer Bošković Institute, Division of Materials Chemistry, Zagreb, Croatia
[4]University of Strathclyde, Strathclyde Institute Of Pharmacy And Biomedical Sciences, Glasgow, United Kingdom

Backgrounds

Streptomycetes are multicellular bacteria that exhibit a complex developmental program and morphological differentiation. The great majority of natural products, including clinnicaly important antibiotics, immunosuppressants and anti-cancer drugs; are synthesized by these bacteria. The tetracyclines are one of the most successful classes of antibiotics that include oxytetracycline (OTC) produced by *S. rimosus*. It has been reported that protein phosphorylation has an important role in the regulation of metabolism and antibiotic production in strepomycetes.

Objectives

Bioinformatic analysis of the *S. coelicolor* genome predicted 47 eukaryotic-like protein kinases and 49 eukaryotic type protein phosphatases in this species. This number of kinases, although larger than other bacteria is not surprising as the average Streptomyces genome size is almost twice that of *E.coli*. Based on the phosphoproteome reported for *S. coelicolor* we predict that the effect of the OTC overproduction in *S. rimosus* is a consequence of alteration in posttranslational modification (PTM) of regulatory and/or OTC biosynthesis proteins.

Methods

Different *S. rimosus* strains were tested for antibiotic production, and analysed using HPLC. Level of phosphorylation during growh was inspected by Western blot and morphology was observed applying viability staining to each strain.

Conclusions

Inspected strains showed great variations in OTC production. Western blot analyses showed different phosphorylation patterns for each strain. Confocal microscopy revealed different morphological features and viability of tested strains.

FEMS7-1762
Physiology / Biochemistry / Molecular Microbiology - Part III

DIFFERENCES IN THE NEONATE MICROBIOME AFTER HOME OR HOSPITAL DELIVERY

J. Combellick[1], S. Hakdong[2], S. Dongjae[3], H. Hagan[1], M.G. Dominguez Bello[4]
[1]New York University, Rory Meyers College of Nursing, New York, USA
[2]Sejong University- South Korea,
College of Life Science- Department of Food Science and Biotechnology, Seoul, Republic of Korea
[3]Sejong University, College of Life Science- Department of Food Science and Biotechnology, Seoul, Republic of Korea
[4]New York University, School of Medicine, New York, USA

Backgrounds

Hospitalization for delivery is considered a foundation of obstetric care. In the U.S. more than 99% of deliveries occur in the hospital. However in many countries—notably those in Scandinavia—home birth is recognized as a safe alternative for low-risk women. Currently there is limited investigation of how the neonatal microbiome develops in the absence of all interventions, including hospitalization. We hypothesize that hospital exposure at birth affects the neonate intestinal microbiota.

Objectives

To characterize the intestinal microbiota of newborns following home and hospital delivery and to investigate differences related to hospitalization during the first month of life.

Methods

A prospective cohort study was conducted to compare breast-fed vaginally delivered infants born at home (n=10) or in the hospital (n=10). Consecutive sampling was performed on infant feces at 7 time points from delivery through day 28. The V4 region of the 16S rRNA gene of 212 samples was sequenced using Illumina MiSeq platform. Sequences were analyzed using the QIIME pipeline.

Conclusions

Hospitalization for birth alters the microbiome of neonates. The gut microbiome differed significantly between home and hospital born neonates (PERMANOVA p=<0.005), with babies born at home showing higher fecal *Bacteroides*, and *Bifidobacterium*, *Streptococcus*, and *Lactobacillus*, at one or more time points after the first week of birth, and lower proportions of *Clostridium* (Day 21), Enterobacteriaceae family (Day 28). More research is needed to determine the health significance of these differences. These results are relevant to health care system policies that support home birth in low-risk women.

FEMS7-1250
Physiology / Biochemistry / Molecular Microbiology - Part III

MEMBERS OF THE CMAX-CFRX-CMPX OPERON ARE DIFFERENTLY REGULATED BY ALGU AND SIGX IN RESPONSE TO HEAT AND COLD SHOCKS IN PSEUDOMONAS AERUGINOSA

E. Bouffartigues[1], I. Si Hadj Mohand[1], D. Tortuel[1], O. Maillot[1], B. Xu[2], A. Bazire[3], A. Dufour[3], D.J. Wozniak[2], N. Orange[1], M.G. Feuilloley[1], P. Cornelis[1], S. Chevalier[1], M. Gallique[1]
[1]University of Rouen Normandy, Laboratory of Microbiology Signals and Microenvironment LMSM, Evreux, France
[2]Center for Microbial Interface Biology- Ohio State University, Departments of Microbial Infection and Immunity- Microbiology, Columbus, USA
[3]Institut Universitaire Européen de la Mer- Université de Bretagne-Sud, Laboratoire de Biotechnologie et Chimie Marines LBCM, Lorient, France

Backgrounds

P. aeruginosa is a highly adaptable opportunistic pathogen, a phenotype that is mainly related to its large number of transcriptional regulators, including 19 extracytoplasmic function (ECF) sigma factors. The well-known RpoE-like ECF sigma factor AlgU is involved in alginate biosynthesis and cell wall stress response. Interestingly, the newly characterized ECF SigX emerged as a second sigma factor responding to envelope stress, controlling in particular membrane composition remodelling. However the functional interactions that may exist between SigX and AlgU to maintain envelope homeostasis remain to be discovered.

Objectives

To this aim, the regulation of a putative operon located directly upstream of *sigX* was studied according to temperature variation, a key parameter affecting the membrane state.

Methods

This predicted operon forms a cluster of three genes, *cmaX-cfrX-cmpX*, among which *cmaX* on one side, and *cfrX* and *CmpX* on the other side, were proposed to belong to AlgU and SigX regulons, respectively. Accordingly, we have identified an internal SigX-dependent promoter region upstream *cfrX*, in addition to the AlgU-dependent promoter region upstream *cmaX*. We show that expression of *cmaX* is increased in response to a heat shock through an AlgU-dependent mechanism that does however not involve RpoH. Remarkably, *cfrX* and *cmpX* expression is up-regulated *via* a SigX-dependent mechanism in response to a cold shock, and it requires also the AlgU-dependent alginate and motility regulator AmrZ, whose expression is partly controlled by SigX in this condition.

Conclusions

Taken together, these data give further insights into the highly complex networks that are involved in maintaining membrane homeostasis in *P. aeruginosa*.

FEMS7-2116
Physiology / Biochemistry / Molecular Microbiology - Part III

LOMENTOSPORA PROLIFICANS RESISTANCE MECHANISMS TO THE ANTIFUNGAL DRUG VORICONAZOLE

A. Pellon[1], L. Martin-Souto[1], I. Buldain[1], A. Antoran[1], A. Rementeria[1], F.L. Hernando[1], A. Ramirez-Garcia[1]
[1]University of the Basque Country UPV/EHU,
Fungal and Bacterial Biomics Research Group. Dept. of Immunology- Microbiology & Parasitology, Leioa, Spain

Backgrounds

The filamentous fungus *Lomentospora* (*Scedosporium*) *prolificans* is an opportunistic pathogen that is intrinsically pan-resistant to available antifungal compounds, including to the first option to treat these mycoses, namely voriconazole. Thus, there is an urgent need for novel truly-effective therapies.

Objectives

To discover the unknown mechanisms involved in the voriconazole resistance in this pan-resistant filamentous fungus.

Methods

To delve into this, we performed an integrated study of *L. prolificans* responses to voriconazole using a wide range of techniques, including fluorescence and electron microscopy to study morphological alterations, ion chromatography to measure changes in cell wall carbohydrate composition, and proteomics-based analyses to identify differentially expressed proteins.

Conclusions

We showed drastic changes occurring in cell shape after voriconazole exposure, *L. prolificans* hyphae being shorter and wider than under control conditions. Interestingly, we proved that the architecture and carbohydrate composition of the cell wall were modified in the presence of the drug. Specifically, *L. prolificans* constructed a more complex organelle with a higher presence of glucans and mannans, among other carbohydrates. In addition, we observed 72 differentially expressed cell surface proteins, being Srp1 and heat shock protein 70 (Hsp70) as the most overexpressed under voriconazole-induced stress conditions. The mechanisms described in this study, which may be related to *L. prolificans* antifungal resistance, could be used as targets to improve existing therapies or to develop new ones against these mycoses.

Funding: GIU15/36 from the Basque Government. AP (postdoctoral), IB and AA (predoctoral) are recipients of fellowships from the UPV/EHU or the Basque Government, respectively.

FEMS7-0303
Physiology / Biochemistry / Molecular Microbiology - Part III

STRUCTURE AND CELL INTERACTION ARE ALTERED IN MEMBRANE VESICLES RELEASED BY A HYPERVESICULATING ESCHERICHIA COLI NISSLE 1917 TOLR MUTANT

C. Perez Cruz[1], M.A. Cañas[2], R. Giménez[2], J. Badía[2], L. Baldomà[2], L. Aguilera[2], E. Mercadé[1]
[1]University of Barcelona, Department of Biology- Healthcare and the Environment, Barcelona, Spain
[2]University of Barcelona, Department of Biochemistry and Physiology, Barcelona, Spain

Backgrounds

Membrane vesicles (MVs) produced by Gram-negative bacteria are being explored for clinical applications. MVs from the probiotic *Escherichia coli* Nissle 1917 (EcN) are good candidates for testing such applications. A drawback is the low level of MV isolation from in vitro culture supernatants, which may be overcome by the use of mutants in cell envelope proteins that yield a hypervesiculation phenotype

Objectives

To confirm that a *tolR* mutation in EcN increases MV production, and to analyze if their composition, ultrastructure and interaction with target cells can be modified by the mutations in the cell envelope proteins.

Methods

EcN *tolR* MVs were analyzed by protein, LPS and fluorescent lipid measurements. Structure was determined by TEM after high-pressure freezing and freeze substitution of bacterial samples, together with cryo-TEM observation of plunge frozen hydrated isolated MVs. Time-course experiments of MV uptake in Caco-2 cells were done using rhodamine- and DiO-labelled MVs.

Conclusions

The *tolR* mutation in EcN induces a hypervesiculation phenotype. MVs showed alterations in composition and in their ability to interact with host cells, which can be explained by significant modifications in MV structure. Production of different types of vesicles by *tolR* mutants cannot be detected by TEM of negatively stained MVs, although this heterogeneity may have a major impact on vesicle functionality. This study evidences the need for conducting a detailed structural analysis by high resolution TEM techniques when working with hypervesiculating mutants. This analysis is crucial to improve and standardize the vesicles used for vaccine and therapy purposes.

FEMS7-0404
Physiology / Biochemistry / Molecular Microbiology - Part III

CHARACTERIZATION OF SMALL RNAS DELIVERED BY MEMBRANE VESICLES FROM PSEUDOMONAS AERUGINOSA PAO1

C. Perez Cruz[1], *E. Mercade*[1]
[1]*University of Barcelona, Department of Biology- Healthcare and the Environment, Barcelona, Spain*

Backgrounds

Membrane vesicles (MVs) are spherical structures (20-200 nm) that are secreted from the membrane of Gram-negative bacteria to deliver bacterial effectors to distant cells. They are implicated in several functions: pathogenesis, horizontal gene transfer and quorum sensing. Recent publications, reported the presence of regulatory small RNA (sRNA) inside MVs although their role it is unknown.

Objectives

The aim of the current work is to identify and determine the function of sRNAs associated with MVs from *Pseudomonas aeruginosa* PAO1.

Methods

RNA and MVs isolation, pico-RNA Bioanalyzer, Qubit RNA HS, RT-qPCR, high-resolution flow cytometry, sRNA RNA-seq, transfer experiments.

Conclusions

To date, we have determined that MVs package sRNAs (25-100 nt) and we confirmed that sRNAs were encapsulated inside MVs through RNase protection assay. By high-resolution flow cytometry, we have quantified MVs during bacterial growth, finding that MVs concentration increases during the transition to stationary phase, while it decreases in the later stationary phase. We have monitored the expression of three PAO1 sRNAs (PhrS, CrcZ and RsmZ) during growth and we found that they were differentially packaged inside MVs. To identify the sRNAs in PAO1 MVs, sequencing of total RNA extracted from MVs obtained at different growth points have been performed. Studies to demonstrate that functional sRNA can be delivered to PAO1 cells by MVs were conducted. The differentially encapsulation of sRNAs inside PAO1 MVs, confirms it is not a random process and opens up to study whether MV-associated sRNAs could play a role in cell-to-cell communication.

FEMS7-1719
Physiology / Biochemistry / Molecular Microbiology - Part III

FUNCTIONAL PROTEINS OF LACTOBACILLUS CASEI BL23 RECOGNIZE SURFACE ELEMENTS OF HUMAN EPITHELIAL CELLS AND REGULATE CELL PROLIFERATION AND INFLAMMATORY RESPONSES

C. Bäuerl[1], M. de la Cruz[2], J.M. Coll[1], M.C. Collado[1], V. Monedero[1], J. Rodriguez Diaz[3], G. Perez Martinez[1]
[1]Instituto de Agroquímica y Tecnología de Alimentos CSIC, Food Biotechnology, Paterna, Spain
[2]Universidad Católica de Valencia, Facultad de Veterinaria y Ciencias Experimentales, Valencia, Spain
[3]Faculty of Medicine, Department of Microbiology, Valencia, Spain

Backgrounds

In order to shed light on probiotic/host interactions, we have studied p40 and p75, two cell wall bound muraminidases (*cmu*A, *cmu*B), from *L. casei* BL23 that contain a conserved C-terminus NLPC/P60 domain for which antiapoptotic and IgA stimulating activity has been reported.

Objectives

To determine differences in the interaction of p40 and p75 with epithelial cells.

Methods

Both proteins bind to the surface of epithelial cells and they are subsequently internalised; however, the binding affinity for extracellular matrix proteins and glycosaminoglucans (GAGs), as determined by surface plasmon resonance (SPR), indicated a different binding profile. p40 showed a very high affinity for heparin and chondroitin sulphate A, suggesting a powerful interaction due to the highly positively charged NH-region of p40. On the other hand, using pNIFTY transfected HT29 cells, both proteins had pro-inflammatory effect, but NF-KB stimulatory effect of p75 was remarkably higher. Protein phosphorylation assays carried out on T-84 epithelial cells confirmed that they activate the epithelial grow factor receptor (EGFR) and *Akt*; however the inducing effect of both proteins may course through alternative metabolic pathways, as demonstrated by the phosphorylation of ERK1/2 intermediate, suggesting that both proteins may interact with different receptors or mediators.

Conclusions

Conclusions:family of NLPC/P60 carrying domain cell surface lysins (muraminidases), their biological properties are different and could be conferred by the amino acid sequences located toward the NH-terminus of the proteins.

FEMS7-2218
Physiology / Biochemistry / Molecular Microbiology - Part III

TRANSCRIPTIONAL RESPONSE OF MUTANTS LACKING THE MRNA DEGRADATION FACTORS XRN1 AND DHH1 DURING OSMOTIC STRESS

M.E. Perez-Martinez[1], D.A. Medina[1], T. Li[1], J.E. Perez-Ortin[1], P. Alepuz[1]
[1]Universitat de Valencia, Facultad de Biologia, Valencia- Burjassot, Spain

Backgrounds

Recent studies strongly suggest that regulation of gene transcription at the nucleus is mechanistically connected, not only with nuclear mRNA processing, but also with cytoplasmic mRNA decay. The crosstalk between degradation and synthesis would serve to maintain proper mRNA levels in the cell. In this context, factors of the mRNA decay machinery, such as the 5'-3' exonuclease Xrn1, have been found to bind chromatin. Under steady-state conditions, deletion of Xrn1 decreases mRNA synthesis and increases mRNA half-life in an approximately compensatory manner.

Objectives

We asked about the role of these crosstalk factors under osmotic stress conditions in which mRNA levels are quickly, profoundly and transiently changed

Methods

We studied the osmotic stress-response in mutants lacking the 5' to 3' mRNA exonuclease Xrn1 and the decapping activator factor Dhh1. For this, we measured the changes in transcription rates, RNA polymerase association to chromatin and mRNA amounts and stability during the stress response at global and mRNA specific levels

Conclusions

Our results show that Xrn1 and Dhh1 are involved in the regulation of the transcription and degradation of up-regulated mRNAs during the response to osmotic stress. Interestingly, although transcription is reduced upon stress in *xrn1Δ* and *dhh1Δ* mutants, we observed high levels of RNA polymerase II binding to osmo-gene promoters. Moreover, the RNA polymerase II bound to osmo-responsive genes appears to be highly phosphorylated. These results suggest that the lack of co-transcriptionally binding of the decaysome to chromatin affects the transcription elongation capacity of the RNA polymerase II. Our results show that the lack of a proper transcriptional induction of osmo-mRNAs in *xrn1Δ* and *dhh1Δ* mutants is compensated by the over-stabilization of the osmo-mRNAs. This result could explain the resistance of *xrn1Δ* and *dhh1Δ* mutants in high osmolarity

FEMS7-3021
Physiology / Biochemistry / Molecular Microbiology - Part III

AN ADH2 GENE CONVERSION DROVE THE ADAPTIVE EVOLUTION OF WINE SACCHAROMYCES CEREVISIAE YEASTS

R. Pérez-Torrado[1], *A. Querol*[1], *E. Barrio*[2]
[1]*Institute of Agrochemistry and Food Technology, Food Biotechnology, Paterna Valencia, Spain*
[2]*Universitat de València, Department of Genetics, Burjassot- Valencia, Spain*

Backgrounds

One of the most important microorganism along human history, *Saccharomyces cerevisiae,* was domesticated to produce wine. However, the key genetic event responsible of its origin is unknown.

Objectives

In this study, we unveiled an *ADH2* allele, derived form an *ADH1-ADH2* gene conversion, which is fixed in wine yeasts and confers a specific metabolic signature.

Methods

This converted Adh2p shows similar ethanol affinity than Adh1p and allows a significant selective advantage in wine fermentations.

Conclusions

Therefore, this study demonstrates that the acquisition of a recombinant *ADH2* allele was a key step in the origin and adaptive evolution of a differentiated wine yeast population.

FEMS7-1684
Physiology / Biochemistry / Molecular Microbiology - Part III

SUBFUNCTIONALIZATION INFLUENCES THE EXPANSION OF BACTERIAL ANTIBIOTIC RESISTANCE SYSTEMS

E. Perrin[1], M. Fondi[1], E. Bosi[1], A. Mengoni[1], S. Buroni[2], G. Riccardi[2], M. Valvano[3], R. Fani[1]
[1]University of Florence, Department of Biology, Sesto Fiorentino Florence, Italy
[2]University of Pavia, Department of Biology and Biotechnology "Lazzaro Spallanzani", Pavia, Italy
[3]Queen's University of Belfast, 3Centre for Experimental Medicine. The Wellcome-Wolfson Institute for Experimental Medicine, Belfast, United Kingdom

Backgrounds

Multidrug resistance (MDR) efflux pumps, in particular those belonging to the RND family, are mainly responsible of antibiotic resistance in Gram-negative bacteria. Their genomes often contain several copies of the different classes of MDR, and the role of gene duplication and the consequent assumption of new functions by the duplicate copies has a key role in the expansion of drug-resistance.

Objectives

The aim is to provide computational and experimental evidence on the evolution and functional diversification of two members of the RND superfamily in *Burkholderia* to understand how these events could have an effect on antibiotic resistance.

Methods

We assessed the conservation and distribution of these two systems together with their regulation mechanisms. This information was then used to design and perform genetic manipulation of these strains aimed at identifying both the exact substrate range of these transporters and their (eventual) interchangeability. Finally, the possible role of antibiotics in the activation of expression of these systems was evaluated, through a direct evolution experiment combined with NGS.

Conclusions

Changes in the regulatory circuit seem to be the first step to diversify the functions of different RND paralogs rather than functional mutations. This agrees with the low substrate specificity of these systems. Moreover, they could also rapidly rewire their regulation to respond to the presence of antibiotics, maintaining a high genomic plasticity. The knowledge of the regulatory network underlying the expression of the RND complexes has important implications in the understanding of the evolution of drug resistant phenotypes and in the fight for their eradication.

FEMS7-1129
Physiology / Biochemistry / Molecular Microbiology - Part III

ALTERNATIVE SCENARIOS OF STARVATION-INDUCED ADAPTATION IN PECTOBACTERIUM ATROSEPTICUM

O. Petrova[1], V. Gorshkov[1,2], I. Sergeeva[3], A. Daminova[1], M. Ageeva[4], Y. Gogolev[1,2]
[1]Kazan Institute of Biochemistry and Biophysics - RAS, Laboratory of Molecular Biology, Kazan, Russia
[2]Kazan Federal University, Botany and Plant Physiology, Kazan, Russia
[3]Kazan Federal University, Genetics, Kazan, Russia
[4]Kazan Institute of Biochemistry and Biophysics - RAS, Laboratory of Microscopy, Kazan, Russia

Backgrounds

Bacteria have high adaptive potential that provides their survival during various environmental challenges. To adapt, bacteria activate a physiological program of stress-response that makes them able to persist under adverse conditions.

Objectives

The aim of this study was to check if the stress-response in a particular bacterial species could be realized according to alternative scenarios depending on the initial physiological status of the cells prior to stress effect.

Methods

As a model, cells of phytopathogenic microorganism *Pectobacterium atrosepticum* at different physiological states (actively growing exponential phase and stationary phase cells) were exposed to starvation and the resulting starving cultures were monitored using CFU counting, quantitative PCR and electron microscopy.

Conclusions

When exponential phase cells were subjected to starvation, the nucleoids of the cells became condensed, and their DNA was detected by qPCR less effectively than the one of the cells growing in nutrient-rich medium or stationary phase cells encountered starvation. Exponential phase cells subjected to starvation were characterized by the increased expression level of genes encoding DNA-binding histone-like proteins. In turn, cell wall deficient forms that were inefficient in their colony forming ability and, thus, had non-culturable phenotype, were formed in the starving cultures inoculated by stationary phase cells. Cell wall deficient forms displayed reduced expression level of genes, encoding synthases of cell-wall components. Our results demonstrated that alternative strategies of adaptation could be employed by *P. atrosepticum* depending on the initial physiological state of bacteria at the time of exposure to stress factor. This study was supported by RFBR 15-04-02380.

FEMS7-1667
Physiology / Biochemistry / Molecular Microbiology - Part III

REGULATION OF K+ HOEMEOSTASIS BY THE NITROGEN PTS (PTS(NTR)) OF PSEUDOMONAS PUTIDA

K. Pflüger-Grau[1], *A. Kremling*[1]
[1]*Systems Biotechnology, Mechanical Engineering, Garching, Germany*

Backgrounds

The nitrogen branch of the phosphotransferase system (PTS^{Ntr}) of *Pseudomonas putida* is a multicomponent regulatory device that participates in controlling a variety of physiological processes in a post-translational fashion. It consists of three proteins PtsP, PtsO, and PtsN, that transfer a phosphate moiety derived from phosphoenol pyruvate. The phosphorylation state of PtsN reflects various metabolic inputs, among them the PEP/Pyr ratio, the presence of fructose, or the nitrogen source.

Objectives

We set out to analyse in more detail the regulatory duties of the PtsN protein in *P. putida*.

Methods

A general survey of the genes regulated by PtsN revealed that transcription of the entire *kdpFABC* operon is influenced by this protein. By monitoring the activity of the *kdpF* promoter in various *pts* mutant strains, we could show that PtsN is responsible for both, repression and activation of the transcription of the *kdpFABC* genes, and that regulation depends on the phosphorylation state of PtsN, in a way that non-phsophorylated PtsN stimulates transcription whereas PtsN~P seems to have the opposite effect. We could show that the regulation is implemented through direct interaction of the PtsN protein with the sensor kinase KdpD of the KdpD/KdpE two-component system. The absence of PtsN led to high KdpFABC expression and reduced growth rates, but also gave rise to suppressor mutations that regained the fast growing phenotype.

Conclusions

Taken together these results suggest that the regulation of K^+-homeostasis is a general duty of the PTS^{Ntr} even though the mechanism does not seem to be conserved between different organisms.

FEMS7-0596
Physiology / Biochemistry / Molecular Microbiology - Part III

REGULATED PROTEOMIC ANALYSIS REVEALS THE EXPORT OF BIOPHARMACEUTICAL PRODUCT VIA TAT PATHWAY IN E. COLI WITH THE EXPRESSION OF CYDISCO PROTEINS

T.K. Pham[1], K. Richards[2], J. Pandhal[1], C. Robinson[2], P.C. Wright[3]
[1]The University of Sheffield, Chemical and Biological Engineering, Sheffield, United Kingdom
[2]University of Kent, School of Biosciences, Canterbury, United Kingdom
[3]Newcastle University, Faculty of Science- Agriculture and Engineering, Newcastle, United Kingdom

Backgrounds

The *E. coli* Sec pathway transports only unfold proteins from the cytoplasm to the periplasm, whereas the TAT system can transport fully folded and assembled proteins. Therefore, we aim to improve/enhance the export of recombinant biopharmaceutical products *via* the TAT pathway by identifying bottlenecks and assessing cell health using a quantitative proteomic approach.

Objectives

Quantitative proteomic analyses were used to understand the responses of *E. coli* during expression of CyDisCo proteins and target biopharmaceuticals (HGH, scFv). Both global and target proteins' regulations were examined to identify key factors to improve the designs of promoters/systems to achieve high yields therapeutic protein production.

Methods

E. coli strains (with/without CyDisCo proteins) were grown on LB medium and collected at 3 h post recombinant protein induction for iTRAQ proteomic analyses performed on a QExactive HF Orbitrap MS (Thermo) coupled with MaxQuant software and our in-house proteomic pipeline for regulated proteome determination.

Conclusions

Of 1,706 quantified proteins, 281 differentially regulated proteins were observed in CyDisCo *E. coli* cells. These regulated proteins belonged to different protein classes and widely involved in a variety of cellular/biological processes. The regulation of some key factors/proteins affecting protein export to the periplasm and degradation of targeted proteins were identified. Furthermore, key proteins relating to various stress responses (induction) such as chaperon/oxidative helpers, sigma factors (eg. E, H, S), *etc.* were also identified. These findings have provided useful information for guiding the specific design of gene promoters and overall optimisation of the host cell system.

FEMS7-1756
Physiology / Biochemistry / Molecular Microbiology - Part III

IDENTIFICATION OF A SUBSTRATE DOMAIN THAT DETERMINES SYSTEM SPECIFICITY IN MYCOBACTERIAL TYPE VII SECRETION SYSTEMS

T. Phan[1], R. Ummels[2], E. Houben[3], W. Bitter[4]
[1]Vrije Universiteit Amsterdam, Molecular Microbiology, Amsterdam, The Netherlands
[2]Vrije Universiteit Medical Center Amsterdam, Medical Microbiology and Infection Control, Amsterdam, The Netherlands
[3]Vrije Universiteit Amsterdam, Molecular Micorbiology, Amsterdam, The Netherlands
[4]Vrije Universiteit Medical Cnter Amsterdam, Medical Microbiology and Infection Control, Amsterdam, The Netherlands

Backgrounds

Type VII secretion (T7S) systems are specialized machineries used by mycobacterial pathogens to transport important virulence factors across their highly hydrophobic cell envelope. There are up to five mycobacterial T7S systems, namely ESX-1 to ESX-5, each of which specifically secretes a different subset of substrates.

Objectives

The T7S substrates or substrate complexes are defined by the general secretion motif YxxxD/E. However this motif does not determine system specificity.

Methods

We have used molecular cloning method to introduce the EspG1 and EspG5 chaperone binding domains into ESX-5 and ESX-1 PPE substrates, respectively. Secretion analysis was performed in different ESX-1 and ESX-5 mutant strains to define effect caused by the swapping of cheprone binding domains.

Conclusions

Here, we show that the substrate domain recognized by the EspG chaperone is the determinant factor for this specificity. We first show that the introduction of point mutations into the $EspG_1$-binding domain of ESX-1 substrate pair PE35/PPE68_1 affects their secretion. Subsequently, we demonstrate that replacing this domain by the $EspG_5$-binding domain of the ESX-5 substrate PPE18 resulted in $EspG_5$ dependence and exclusive rerouting to the ESX-5 system. This rerouting of PE35/PPE68_1 to the ESX-5 system had a negative effect on the secretion of endogenous ESX-5 substrates.

FEMS7-0901
Physiology / Biochemistry / Molecular Microbiology - Part III

BIOFILM FORMATION BY CLOSTRIDIUM LJUNGDAHLII IS INDUCED BY SODIUM CHLORIDE: TRANSCRIPTOME ANALYSIS

J. Philips[1], K. Rabaey[1], D.R. Lovley[2], M. Vargas[3]
[1]Ghent University, Center for Microbial Ecology and Technology CMET, Ghent, Belgium
[2]University of Massachusetts, Department of Microbiology, Amherst, USA
[3]College of the Holy Cross, Department of Biology, Worcester, USA

Backgrounds

The acetogen *Clostridium ljungdahlii* is capable of syngas fermentation and microbial electrosynthesis. Both these applications could benefit from biofilm formation. We demonstrated before that *C. ljungdahlii* biofilm formation is induced by the addition of sodium chloride to the medium. The molecular mechanisms behind biofilm formation by *C. ljungdahlii*, however, remain to be unraveled.

Objectives

A transcriptome analysis was performed to compare the gene expression of planktonic (no NaCl) and biofilm (NaCl addition) cells of *C. ljungdahlii*.

Methods

The addition of NaCl clearly induced stress to the cells, as the general stress response genes were upregulated. In addition, *C. ljungdahlii* coped with the salt stress by the upregulation of Na^+ export and osmoprotectant accumulation. Flagellar motility was downregulated in the biofilm cells, while putative type IV pili biosynthesis genes were not expressed in both conditions. The D-alanine pathway was strongly upregulated and is potentially involved in giving a positive charge to cell wall techoic acids to improve attachment, similarly as in *Staphylococcus* species. In addition, the biosynthesis of UDP-N-acetylglucosamine was upregulated, which is the precursor for poly-N-acetylglucosamines, the extracellular polysaccharides potentially responsible for biofilm formation, as for *Bacillus* and *Staphylococcus* species. Moreover, the gene expression analysis suggested the involvement of the transcriptional regulators LexA, Spo0A and CcpA in the stress response and biofilm formation.

Conclusions

This work is the first to gain insight into the regulation and molecular mechanisms of *C. ljungdahlii* stress response and biofilm formation.

FEMS7-1061
Physiology / Biochemistry / Molecular Microbiology - Part III

CHARACTERISTICS OF MOLECULAR-GENETIC STRUCTURE OF PHAGE PF-10

T. Pilipchuk[1], L. Valentovich[2], M. Titok[1], E. Kolomiets[1]
[1]Institute of Microbiology - Belarus National Academy of Sciences, Laboratory of Biological Control Agents, Minsk, Belarus
[2]Institute of Microbiology - Belarus National Academy of Sciences, Laboratory "Center of Analytical and Genetic Engineering Research", Minsk, Belarus

Backgrounds

Bacteriophages capable to lyse pathogenic bacteria constitute active principle of antimicrobial preparations and make an alternative to antibiotics. Search and characterization of bacteriophages lays the basis for their application directed at formulation of biological agents controlling microbial pathogens.

Objectives

Aim of study was to analyze genome nucleotide sequence of bacteriophage Pf-10 - key ingredient of biopesticide Multiphage owing to ability to lyse phytopathogenic bacteria of genus *Pseudomonas*.

Methods

Various microbiological and molecular-genetical methods were used: isolation of phage DNA, PCR, sequencing, annotation of genome.

Conclusions

The conducted investigations resulted in deciphering of full nucleotide sequence of phage Pf-10 isolated from common bean leaves in Belarus (GenBank registration number NCBI KP025626).

Analysis of full nucleotide sequence of phage Pf-10 genome allowed to refer this linear double-stranded DNA virus to T7 group of genus *Autographivirinae*, family *Podoviridae*, order *Caudovirales.*

Genome sequencing of phage Pf-10 has revealed 46 open reading frames determining synthesis of proteins governing stages of lytic cycle. The genes and encoded proteins showed the closest similarity to homologous nucleotide and amino acid sequences of phage phiIBB-PF7A infecting bacteria *Pseudomonas fluorescens* and phage Phi-S1 displaying a broad host specificity toward *Pseudomonas* bacteria. In particular, amino acid sequences of DNA-dependent RNA polymerase, DNA polymerase, amidase, primase, helicase, endo- and exonuclease, phage tail proteins are 98-99% identical with homologous polypeptides of phage Phi-S1, while DNA ligase, inhibitor of bacterial RNA polymerase, endopeptidase, capside proteins showed 90-99% identity with functionally related proteins of phage phiIBB-PF7A, evidencing mutational modifications occurring in the course of phage Pf-10 genome evolution.

FEMS7-2180
Physiology / Biochemistry / Molecular Microbiology - Part III

UNWIRING HELICOBACTER PYLORI TRANSCRIPTIONAL REGULATORY NETWORK INVOLVED IN METAL HOMEOSTASIS CONTROL THROUGH CHIP-SEQ AND RNA-SEQ DATA INTEGRATION

E. Pinatel[1], A. Vannini[2,3], P.E. Costantini[2], S. Pelliciari[2], D. Roncarati[2], S. Puccio[1,4], G. De Bellis[1], A. Danielli[2], C. Peano[1]
[1]National Research Council, Institute for Biomedical Technologies, Segrate Milan, Italy
[2]University of Bologna, FaBiT - Department of Pharmacy and Biotechnology, Bologna, Italy
[3]University of Bologna, DIMES Department of Experimental- Diagnostic and Specialty Me dicine, Bologna, Italy
[4]University of Milan, Doctoral School of Molecular and Translational Medicine, Milan, Italy

Backgrounds

Genome-wide approaches are becoming crucial to provide system-level comprehension of bacterial species and to obtain a landscape view of their transcriptional regulatory network. *Helicobacter pylori* is a human pathogen surviving in the prohibitive gastric environment where it can cause cancer. Its genome encodes for only 17 transcription factors (TF) whose rapid and fine-tuned expression orchestrates *H. pylori* ability to counteract environmental stresses and to perstist in the stomach despite host defence responses. The metal-dependent regulators NikR and Fur directly control metal ion homeostasis in *H. pylori* and indirectly impact on several processes involved in its virulence and survival.

Objectives

The aim of this work was to characterize the complete regulons of these two TFs, characterizing their putative cooperation or antagonism towards common binding sites.

Methods

ChIP-seq and RNA-seq experiments were performed in parallel on wild-type *H. pylori* G27 strain and on the knockout strain for each TF, both in standard growth conditions and in conditions inducing or repressing their activity. The results of ChIP-seq and RNA-seq studies were integrated to discriminate transcriptionally active DNA binding sites and, finally, network analysis was adopted to reveal Fur and NikR interaction on common binding sites.

Conclusions

Our study identifies new and known targets of Fur and NikR, improving the characterization of their biological role. Moreover it sheds light on the entire framework of dynamic interactions occurring between these TFs and between each TF and its regulatory targets. This integrated view can help in unraveling new strategies to counteract the persistence and pathogenicity of *H. pylori*.

FEMS7-1290
Physiology / Biochemistry / Molecular Microbiology - Part III

LARS CONTROLS THE EXPRESSION OF THE AGR SYSTEM OF LISTERIA MONOCYTOGENES AND MEDIATES VIRULENCE

J. Pinheiro[1,2], A. Carreaux[1,3], F. Carvalho[1,2], S. Sousa[1], D. Cabanes[1]
[1]IBMC – Institute For Molecular and Cell Biology- i3S - Institute for Research and Innovation in Health , Group of Molecular Microbiology, Porto, Portugal
[2]University of Porto, ICBAS- Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal
[3]Université Paris Diderot-Paris 7, SDV - UFR Sciences Du Vivant, Paris, France

Backgrounds

To succeed as a pathogen, *Listeria monocytogenes* depends on the ability to cross physiologic barriers, invade diverse host cells and avoid phagocytosis by macrophages. Adaptation to the host environment is a critical factor that requires mechanisms of highly coordinated expression of virulence factors. We provided the first comprehensive view of the genome expression of a pathogen in deeper organs of infected mice, where we demonstrated that the analysis of genes differentially expressed during infection is a powerful tool for the identification of new virulence factors otherwise difficult to predict[1]. We observed that two major virulence regulators (PrfA/VirR), as well as their downstream effectors, were up regulated *in vivo*. However, other putative regulator-encoding genes that could be important for virulence are also differentially expressed by *L. monocytogenes in vivo*.

Objectives

To identify and characterize novel virulence regulators of the pathogen *L. monocytogenes.*

Methods

Through extensive analysis of our transcriptomic data and mutagenesis, we identified a novel regulator LarS involved in *L. monocytogenes* virulence*. In vitro* cell invasion assays and *in vivo* mice infections were performed to study the roles of LarS in *L. monocytogenes* virulence. An RNAseq transcriptomic approach was developed to characterize its regulatory network.

Conclusions

The Δ*larS* mutant shows impaired ability to invade specific human cell lines and shows defects in mouse infection. We identified the Agr System genes (*agrBDCA*) as targets of *larS* positive regulation and demonstrated how *L. monocytogenes* expresses this regulator to modulate chitinase activity and biofilm formation in order to achieve full virulence.

1.Camejo A. etal. PLoS Pathog, 2009

FEMS7-2185
Physiology / Biochemistry / Molecular Microbiology - Part III

NOVEL DRUG DISCOVERY PLATFORM BASED ON TARGETING SENSORY PATHWAYS OF STAPHYLOCOCCUS AUREUS

J.M. Prieto[1], *D. Viana*[2], *J.R. Penadés*[3], *I. Lasa*[4], *C. Latasa*[5]
[1]*RecombinaSL, I+D, Mutilva, Spain*
[2]*Universidad Cardenal Herrera, Producción y Sanidad Animal, Valencia, Spain*
[3]*University of Glasgow, Institute of Infection Immunity and Inflammation, Glasgow, United Kingdom*
[4]*Navarrabiomed-Universidad Pública de Navarra-IdiSNA, Direction, Pamplona, Spain*
[5]*RecombinaSL, Direction, Mutilva, Spain*

Backgrounds

Biotech and pharmaceutical sectors are constantly seeking novel therapeutic approaches that might deliver us from a future where infections by resistant bacteria could pose a greater threat to human health than cancer itself. One of these strategies consists of targeting the bacterial signalling pathways known as Two Component Systems (TCSs). These systems synchronize gene expression with the different environments that bacteria might encounter, and thus regulate key biological traits as virulence or biofilm formation. Unlike the conventional antibiotics, which target essential functions, anti-TCS drugs disarm pathogens and let the immune system kill them, reducing the tendency to generate resistance and leading to less side effects towards neutral and beneficial bacteria.

Objectives

The main goal of our project is to develop a TCS-targeted drug-screening platform that might contribute to discover novel antibacterial compounds.

Methods

With a starting point of a unique *Staphylococcus aureus* strain in which all the 15 non-essential TCSs had been deleted, and upon deciphering the critical role of one of fifteen non-essential staphylococcal TCS in vivo, several reporter strains based in luminescence and fluorescence emission have been constructed.

Conclusions

The collections of reporter Staphylococcal strains developed in this project represent a promising tool for drug-discovery in functional and reporter screening assays.

FEMS7-0571
Physiology / Biochemistry / Molecular Microbiology - Part III

CHARACTERISATION OF THE "VIABLE BUT NOT CULTIVABLE" STATE TRANSITION IN FOUR VIBRIO SPECIES

E. Prosdocimi[1,2], Z. Zeaiter[2], S. Arioli[2], F. Mapelli[2], E. Crotti[2], S. Borin[2]
[1]Queen Mary University of London, Blizard Institute, London, United Kingdom
[2]Università degli Studi di Milano,
Department of Food- Environmental and Nutritional Sciences DeFENS, Milan, Italy

Backgrounds

Vibrios are marine bacteria able to enter the Viable But Not Cultivable (VBNC) state. VBNC cells are no longer cultivable on plates, unless after a "resuscitation" process. VBNC transition was previously described in different *Vibrio* species but no direct comparison between them was tempted. H_2O_2 has a key role in the VBNC behaviour in *V. vulnificus*, but it is unclear if this is a universal mechanism.

Objectives

To characterise the morphological changes in four *Vibrio* strains during the VBNC transition and resuscitation, investigating in particular the role of oxidative stress.

Methods

Vibrio harveyi BAA-1117™*, V. fortis* UU24*, V. hepatarius* UU21 and *V. nereis* M5 cells were incubated in seawater at 4°C for 50 days. Their size, viability and cultivability were monitored by flow cytometry combined with the LIVE/DEAD-BacLight-kit and by plating. The first two strains became VBNC, and were resuscitated by incubation at 30°C. To assess H_2O_2 sensitivity, catalase-supplemented plates or broths containing different H_2O_2 concentrations were used.

Conclusions

Even though only two strains lost cultivability, all of them displayed cell dwarfing, decrease in DNA quantity and appearance of a peculiar fluorescence upon staining with propidium iodide. These features were partially or totally reverted with resuscitation. Cultivability was partially rescued by plating on catalase-supplemented media. VBNC cells of BAA-1117™ and UU24 were permanently damaged by 0.007 or 0.02 mM H_2O_2, respectively. Conversely, resuscitating cells gradually increased their resistance to H_2O_2. The details of the processes differed among strains. Therefore we suggest that there is no unique “VBNC-pathway", even within the same bacterial genus.

FEMS7-2286
Physiology / Biochemistry / Molecular Microbiology - Part III

REFINEMENT OF THE REGULON OF THE PSEUDOMONAS AERUGINOSA VIRULENCE REGULATOR ΣVREI BY RNA-SEQ

J.M. Quesada[1], J.R. Otero-Asman[1], C. Civantos[1], L. Alted-Pérez[1], M.A. Llamas[1]
[1]Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas, Environmental Protection, Granada, Spain

Backgrounds

Bacterial gene expression is controlled by modifying the promoter affinity of the RNA polymerase (RNAP). This control occurs through the substitution of the RNAP σ subunit and by the interaction with transcription factors. The pathogen *Pseudomonas aeruginosa* contains several σ factors, including σ^{Vrel}. This protein belongs to the extracytoplasmic function (ECF) group of the σ^{70} family. Expression and activity of σ^{ECFs} are tightly regulated and only occur in response to specific signals. σ^{Vrel} expression occurs in response to inorganic phosphate (Pi) limitation and requires the PhoB transcription factor. σ^{Vrel} activity is modulated by both the VreR anti-sigma factor, which keeps σ^{Vrel} inactive in absence of the inducing signal, and by PhoB. Upon activation of the cascade, likely in response to a still unknown host signal, VreR is proteolytically degraded and σ^{Vrel} released. σ^{Vrel} then interacts with the RNAP and with PhoB, and this transcription complex targets the expression of the σ^{Vrel} regulon, which includes virulent functions that increase *P. aeruginosa* pathogenicity.

Objectives

The σ^{Vrel} regulon was first identified by microarray of cells overexpressing σ^{Vrel}, a situation in which extremely high levels of σ^{Vrel} are produced bypassing some of the requirements needed *in vivo* for the σ^{Vrel}-mediated transcription. In this work we have refined the σ^{Vrel} regulon by RNA-seq upon activation of σ^{Vrel} in conditions that resemble the *in vivo* situation, *i.e.* Pi starvation and a *vreR* mutant to reach maximal σ^{Vrel} activity.

Methods

RNA-seq

Conclusions

Several potential new σ^{Vrel}-regulated genes have been identified, providing new insights into this important signaling cascade.

FEMS7-1648
Physiology / Biochemistry / Molecular Microbiology - Part III

TRANSLATIONAL-REPRESSIVE REGULATION DURING IRON DEFICIENT CONDITIONS BY THE MRNA-BINDING PROTEIN CTH2 IN SACCHAROMYCES CEREVISIAE

L. Ramos-Alonso[1], A.M. Romero[1], M.À. Soler[2], P. Alepuz[3], S. Puig[1], M.T. Martínez-Pastor[2]
[1]Instituto de Agroquímica y Tecnología de Alimentos IATA-CSIC, Biotechnology Department, Paterna- Valencia, Spain
[2]Universitat de València, Departamento de Bioquímica y Biología Molecular, Burjassot- Valencia, Spain
[3]Universitat de València, Departamento de Bioquímica y Biología Molecular and ERI Biotecmed, Burjassot- Valencia, Spain

Backgrounds

Iron is an essential micronutrient and a valuable cofactor for its redox properties, although its bioavailability is frequently limited. Under iron deficient conditions, *Saccharomyces cerevisiae* activates the transcription of a group of genes collectively known as the iron regulon, which includes *CTH2*. Cth2 belongs to the tristetraprolin family of proteins that specifically interact through two $Cx_8Cx_5Cx_3Hx_{18}Cx_8Cx_5Cx_3H$ tandem zinc-fingers (TZF) with adenosine/uridine-rich elements (AREs) within the 3'-untranslated region of multiple mRNAs, usually related to iron-dependent pathways, to promote their degradation. Cth2-dependent regulation allows the proper redistribution of iron within the cell to optimize its utilization in essential processes.

Objectives

We aim to study whether Cth2 functions as a translational repressor of ARE-containing mRNAs during iron deficiency. We will determine the contribution of Cth2 and the AREs to the translational regulation of specific mRNAs, find out the region of Cth2 responsible of this regulation and discover possible new target mRNAs and molecular mechanisms implicated in this process.

Methods

The translation efficiency of Cth2-target mRNAs will be determined by two methods: (1) quantitation of the mRNA and protein levels of specific mRNAs, and (2) polysome profile analyses of the mRNAs associated with monosomes vs. polyribosomes.

Conclusions

In addition to promote mRNA decay, Cth2 represses the translation of multiple of its target mRNAs in response to iron deficiency. Both Cth2 TZFs and AREs within the target mRNA are essential for decay as well as for translational repression. Presumably, Cth2 acts as a translational repressor due to its interaction with a translation-repressive machinery through its carboxy-terminal region.

FEMS7-0627
Physiology / Biochemistry / Molecular Microbiology - Part III

CSRA/RSMA FAMILY PROTEINS NEGATIVELY AFFECT GLOBAL C-DI-GMP POOLS AND BIOFILM FORMATION IN PSEUDOMONAS PUTIDA BY REGULATING CFCR

Ó. Huertas-Rosales[1], M. Romero[2], S. Heeb[2], M. Cámara[2], M. Espinosa-Urgel[1], M.I. Ramos-González[1]
[1]Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas, Environmental Protection, Granada, Spain
[2]University of Nottingham, Life Sciences, Nottingaham, United Kingdom

Backgrounds

CfcR is a response regulator with an active GGDEF domain in *Pseudomonas putida*. Previous work has revealed that *cfcR* expression is regulated by RpoS, ANR and FleQ, and the functionality of the protein as a diguanylate cyclase requires an active multi-sensor (CHASE3/GAF) hybrid histidine kinase named CfcA.

Objectives

Our aims were i) to investigate an additional degree of regulation in *cfcR* expression by the three small RNA-binding proteins of the CsrA/RsmA family present in *P. putida*; ii) to evaluate the contribution of CfcR to the free pool of c-di-GMP and the augmented biofilm phenotype exhibited by a triple mutant with *rsmA*/*rsmE*/*rsmI* deleted (*ΔrsmIEA*).

Methods

Specific binding of the three Rsm proteins to *cfcR* mRNA, containing the leader sequence and initiation of translation, was analyzed using fluorescence-based gel shift assays. To evaluate gene expression and quantify the free pool of c-di-GMP “in vivo” we used gene fusions to reporter genes. Microtitter plate assays were used for biofilm quantification.

Conclusions

RsmA bound *cfcR* RNA with the highest affinity but deletion of *rsmA* alone, similarly to the single *rsmE* or *rsmI* deletions, did not cause *cfcR* induction, which was patent in the triple mutant *ΔrsmIEA*. CfcR is responsible for much of the free pool of c-di-GMP during the stationary phase, whose major depletion was caused by RsmA when it remained as the only Rsm protein in the mutant *ΔrsmIE.* A boost of c-di-GMP was observed in *ΔrsmIEA* and was dependent on CfcR, which is a key player for Rsm-controlled biofilm formation in *P. putida*.

FEMS7-0582
Physiology / Biochemistry / Molecular Microbiology - Part III

PROTEOME CHARACTERIZATION OF THE WHOLE TWO-COMPONENT SYSTEM NETWORK IN STAPHYLOCOCCUS AUREUS

B. Rapún[1], B. García[1], J. Valle[1], J. Fernández-Irigoyen[2], X. Martínez[2], E. Santamaría[2], C. Solano[1], I. Lasa[1]
[1]Navarrabiomed.Universidad Pública de Navarra, Dpto. de Salud, Pamplona, Spain
[2]Navarrabiomed.Universidad Pública de Navarra, Unidad de Proteómica, Pamplona, Spain

Backgrounds

Bacteria use two-component systems (TCSs) to sense and respond to environmental changes. Each TCS senses an array of environmental stimuli and the number of TCS in a bacterial genome seems to be proportional to the amount of environments it encounters during life cycle. Current knowledge about TCS have been inferred from the analysis of recombinant bacteria that lack individual members, yet we know little about the regulated proteins by each individual TCS. Here, we use a collection of *S. aureus* strains containing an individual TCS to determine the sub-proteome of every TCS without interferences from other members of the family.

Objectives

To identify the sub-proteome that specifically depends on each TCS of *S. aureus*.

Methods

Protein extracts from *S. aureus* strains containing an individual TCS were prepared under the same growing conditions (TSB, 37ºC OD 0,8), and label-free differential proteomic assay was carried out. Results were compared with the protein extracts from the wild type and the mutant strain in all non-essential TCSs. Bioinformatics analysis identified the group of proteins differentially regulated by each TCS and specific proteomes have been validated using reporter systems.

Conclusions

We identified and validated the sub-proteome of each TCS of *S. aureus*. Our results indicate that the number of proteins specifically regulated by each TCS in a condition is low, with little overlapping between sub-proteomes. Results suggest that TCS network is organized as self-sufficient modules, each of them conferring the capacity to adapt to a different environmental conditions.

FEMS7-2163
Physiology / Biochemistry / Molecular Microbiology - Part III

CLASSIFICATION OF ISOLATES FROM THE PSEUDOMONAS FLUORESCENS COMPLEX INTO PHYLOGENOMIC GROUPS BASED IN GROUP-SPECIFIC MARKERS

D. Garrido-Sanz[1], E. Arrebola[1], F. Martínez-Granero[1], S. García-Méndez[1], C. Muriel[1], E. Blanco-Romero[1], M. Martín[1], R. Rivilla[1], M. Redondo-Nieto[1]
[1]Universidad Autónoma de Madrid, Biology, Madrid, Spain

Backgrounds

The *Pseudomonas fluorescens* complex of species includes plant-associated bacteria with potential biotechnological applications in agriculture and environmental protection. Many of these bacteria can promote plant growth by different means, including modification of plant hormonal balance and biocontrol. The *P. fluorescens* group is currently divided into eight subgroups in which these properties and many other ecophysiological traits are phylogenetically distributed.

Objectives

To design a fast and cheap PCR based technique to obtain a rapid phylogroup assignation from any environmental sample

Methods

Using comparative genomics on 71 *P. fluorescens* genomes, we have identified nine markers which allow classification of any isolate into these eight subgroups, by a presence/absence PCR test. Primer pairs were developed for the amplification of these markers. Their specificity and were assessed on 28 field isolates, environmental samples from soil and rhizosphere and tested by *in silico* PCR on 421 genomes. Phylogenomic analysis validated the results: this PCR-based classification method has a 98.34% of accuracy.

Conclusions

Considering the phylogenetic distribution of biotechnology relevant traits, this assay could provide a new tool for Pseudomonads screening with putative biotechnological applications. Thus, this fast and cheap method could be one of the first steps in protocols that require the screening of many pseudomonads´ isolates to evaluate the potential of any *P. fluorescens* complex strain before going further on genomics analysis.

Research was funded by grant BIO2015-64480R from MINECO/FEDER EU. DGS was granted by FPU fellowship program (FPU14/03965) from MECD Spain. EBR is granted by a fellowship from Fundación Tatiana Pérez de Guzmán el Bueno, Spain.

FEMS7-1444
Physiology / Biochemistry / Molecular Microbiology - Part III

PHENOTYPIC AND GENOTYPIC IDENTIFICATION OF URINARY TRACT INFECTIONS CAUSED BY EXTENDED-SPECTRUM-BETA-LACTAMASE PRODUCING E. COLI IN THE LEICESTERSHIRE AREA

R. Reid[1]
[1]*De Montfort University, Health and Life Sciences, Leicester, United Kingdom*

Backgrounds

Uropathogenic *E. coli* is one of the highest producers of ESBLs (Extended-spectrum β-lactamases), a major public health concern.

Objectives

This study aims to investigate the prevalence of ESBLs and plasmid types in Leicestershire.

Methods

380 urinary *E.* coli ESBL producing isolates were collected from the Leicester Royal Infirmary. Phenotypic analysis involved the MAST ESBL detection kit. Genotypic prevalence was investigated by using a multiplex PCR assay. Primers for blaCTX-M, blaSHV, blaOXA and blaTEM were designed. A second multiplex PCR assay was designed to identify blaCTX-M-1, blaCTX-M-2, blaCTX-M-8, blaCTX-M-9 and blaCTX-M-25. To investigate the relationship between plasmid type and ESBLs, a multiplex PCR-based replicon typing assay was designed[RR1] to detect IncFIA, IncI1, IncL/M, IncN and IncFII.

Conclusions

Phenotypic analysis showed blaCTX-M genes confer higher resistance to CTX, than CAZ and CPD. All isolates that showed resistance to CAZ and CPD, were also resistant to CTX. Prominence for ESBL genes was blaCTX-M (37%)> blaOXA (5%), blaTEM and blaSHV (2%). Associations between the ESBL groups were detected. In the second multiplex assay, the most prominent were blaCTX-M-1 (56%) and blaCTX-M-9 (11%), with associations between the blaCTX-M groups detected. blaCTX-M was found to be associated with all replicons other than L/M.

This is the first study to analyse the prevalence of uropathogenic ESBLs in Leicestershire. blaCTX-M is the most prominent ESBL in Leicestershire. blaCTX-M can be associated with other ESBLs. blaCTX-M-1 is the most common subgroup. Multiple associated plasmids can increase the spread of resistance, causing the epidemic we see today.

FEMS7-2976
Physiology / Biochemistry / Molecular Microbiology - Part III

SUBTILIN CHALLENGE INDICATES CROSS-REGULATION OF BCE-LIKE MODULES IN LACTOBACILLUS CASEI

A. Revilla Guarinos[1], C. Alcántara Baena[2], S. Gehbard[3], T. Mascher[1], M. Zúñiga[2]
[1]Technische Universität Dresden- Institute of Microbiology, 'General Microbiology, Dresden, Germany
[2]Instituto de Agroquímica y Tecnología de Alimentos IATA-CSIC, Biotecnología de Alimentos, Valencia, Spain
[3]University of Bath, Department of Biology and Biochemistry, Claverton Down, United Kingdom

Backgrounds

Lactobacillus casei is a probiotic lactic acid bacterium exposed to environmental stresses such as antimicrobial peptides (AMPs). BceRSAB-like modules mediate resistance against AMPs in some Firmicutes bacteria. BceRSAB modules contain a signal transduction Two Component System (TCS), BceRS, functionally associated to an ABC transporter, BceAB. *L. casei* BL23 possesses two paralogous BceRSAB modules involved in AMP resistance, Module09 and Module12. In response to nisin, TCS09 induces the expression of the ABC09 whereas module 12 acts as a sensory system that regulates the expression of genes involved in controlling cell surface properties (the *dlt* operon and the *mprF* gene) as well as an orphan ABC transporter. Maintaining specificity in TCS signaling is often critical for survival. The protein sequence identity of HK09 - HK12, and RR09 - RR12 is 37% and 44%, respectively.

Objectives

In the current study, we delve in the function and regulation of the paralogous Module09 and Module12 aiming to determine if cross-regulation is possible between these highly similar signaling pathways.

Methods

To address these questions we created a collection of single and double mutants deficient in different combinations of Bce-like elements. These mutants were phenotypically characterized in response to AMPs and the expression of the genes of interest was monitored by RT-qPCR after nisin or subtilin challenges. We also performed bacterial two-hybrid assays to test protein interaction between non-cognate partners from both modules.

Conclusions

Our results indicate that cross-regulation between Module09 and Module12 is possible at least at the level of RR-promoter interaction, under the conditions tested.

FEMS7-2428
Physiology / Biochemistry / Molecular Microbiology - Part III

ANTIBIOTIC SUSCEPTIBILITY AND VANCOMYCIN RESISTANCE OF STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM PATIENTS IN IRAN

V. Alekhamis[1], S.E. Rezatofighi[1], H. Motamedi[1]
[1]Shahid Chamran University of Ahvaz, Biology, Ahvaz, Iran

Backgrounds

Empiric therapy for staphylococcal infections by penicillins led to spread of penicillin resistance throughout the world**.** Treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming complicated due to the formation of new antibiotic resistance.

Objectives

In this study, the antibiotic and vancomycin susceptibility pattern of *S. aureus* isolates was investigated by the phenotypic and molecular methods.

Methods

A total of 100 *S. aureus* isolates were collected from clinical specimens of patients in hospitals of Khuzestan province. These isolates were checked for the presence of vancomycin resistance genes (*vanA, vanB, vanC,* and *vanD*) by PCR. Phenotypic vancomycin-resistance pattern of isolates was checked by minimum inhibition concentration (MIC) and agar dilution methods. Also *S. aureus* isolates were investigated for resistance to various antibiotics by disc diffusion method.

Conclusions

Among the isolates, *vanA*, *vanB*, *vanC*, and *vanD* genes were found in 1, 0, 1, and 1 isolates respectively. The isolate that harbored *vanA* gene showed vancomycin resistance equal to 16 µg/ml; therefore, it was considered VRSA. Two other isolates that showed *vanC* and *vanD* genes, expressed resistance to vancomycin up to concentrations of 8 µg/ml and 4 µg/ml respectively. These isolates considered vancomycin-intermediate *S. aureus* (VISA). High resistance profiles were observed to penicillin (99%), followed by cefoxitin (95%), tetracycline (90%), kanamycin (87%), ciprofloxacin (78%), and amikacin (72%). In conclusion, detection of VRSA in this study emphasizes that careful surveillance and monitoring of vancomycin resistance with staphylococcal infections is needed.

FEMS7-0038
Physiology / Biochemistry / Molecular Microbiology - Part III

STRUCTURAL BASIS OF QUORUM SENSING-DEPENDENT OXALATE PRODUCTION

J. Oh[1], S. Rhee[1]
[1]Seoul National University, Agricultural Biotechnology, SEOUL, Republic of Korea

Backgrounds

Bacterial quorum sensing (QS), a cell-to-cell communication process in many *Proteobacteria,* controls the gene expression for bacterial population-wide characteristics, including bioluminescence, motility, and virulence-related factors. Recently, QS has been recognized to provide further benefits at the population level by regulating the production of public goods, the function of which could be beneficial to all members of the group. In *Burkholderia* species, oxalic acid was recently identified as an excreted public good for the QS-dependent growth. In these species, QS-mediated oxalogenesis via the oxalate biosynthetic component (obc) is a cellular event indispensable for the survival of bacteria in the stationary phase. In *B. glumae*, obc consists of two genes encoding ObcA and ObcB for coordinating the production of oxalic acid, as well as acetoacetate and CoA, by using oxaloacetate and acetyl-CoA as substrates. Unlike *B. glumae*, there is a bi-functional enzyme Obc1 from *B.thailandensis.* Sequence analysis indicates that Obc1 has an ObcA-like N-terminal domain and shows ObcB activity in its C-terminal domain despite no sequence homology with ObcB.

Objectives

We are to understand structural and functional features of Obc1.

Methods

X-ray crystallography and enzyme kinetics were employed to investigate the catalytic residues and a possible mechanism of oxalate production.

Conclusions

In Obc1, the C-terminal domain has an α/β hydrolase fold that has a catalytic triad for oxalate production and a novel oxyanion hole distinct from the canonical HGGG motif in other α/β hydrolases. Functional analyses through mutagenesis studies suggested that His-934 is an additional catalytic acid/base for its lyase activity and liberates two additional products, acetoacetate and CoA. These results provide structural and functional insights into bacterial oxalogenesis. This work was supported by Next Generation BioGreen 21 program of Rural Development Administration (Plant Molecular Breeding Center) of Republic of KOREA.

FEMS7-2250
Physiology / Biochemistry / Molecular Microbiology - Part III

INVESTIGATION OF LIPOPROTEINS TRANSLOCATION SYSTEM IN N. MENINGITIDIS

B. Ricchetti[1], M. Giuliani[1], M. Scarselli[2], V. Scarlato[3], I. Delany[1]
[1]GSK vaccine, AIMB, Siena, Italy
[2]GSK vaccine, Proteomic and antigen structure, Siena, Italy
[3]University of bologna, Pharmacy and biotechnology, Bologna, Italy

Backgrounds

Lipoproteins (LPs) of pathogenic Gram-negative bacteria are involved in different biological processes. For some Gram-negative bacteria, like *E. coli* (Ec), the lipoprotein translocation machinery is well characterized and, for *N. meningitidis* (*Nm*), homologues of these proteins can be identified from the genome. *Nm*, unlike *Ec*, displays many lipoproteins on its surface suggesting the presence of unknown factors involved in this mechanism. An additional translocation component, Surface lipoprotein assembly modulator (Slam), involved in the surface exposure of specific *Nm* lipoproteins has been recently identified.

Objectives

In this work we investigate the surface lipoproteins (SLPs) translocation mechanism mediated by Slam in different *Nm* strains and in heterologous systems.

Methods

We analyzed the surface exposure of NHBA (Neisserial-Heparin-Binding-Antigen) and fHbp (factor-H-binding-protein) as representatives of *Nm* SLPs by FACS. SLPs expression was confirmed by WB analysis of whole-cell lysate. We tested heterologous surface expression of *Nm* SLPs in the *E. coli (Ec)* background by generating expression plasmids carrying SLP and Slam.

Conclusions

Our results validated the role of Slam in outer membrane translocation mechanism in *Nm*. Slam was necessary for surface exposure of NHBA and had an impact in fHbp protein expression/stability in *Nm.* Co-expression of fHbp with functional Slam protein in *Ec* resulted in significantly higher levels of fHbp expression and its surface exposure.
In conclusion, we confirm the role of Slam in SLPs surface localization, furthermore our results suggest that Slam may play a differential role in the correct surface assembly of lipoproteins, depending on the nature of the lipoprotein.

FEMS7-1681
Physiology / Biochemistry / Molecular Microbiology - Part III

DESCRIPTION OF THE FTSZ DISASSEMBLY PHENOTYPE

A.I. Rico[1], *D. López-Escarpa*[1], *M. Krupka*[1], *M. Vicente*[1]
[1]*Centro Nacional de Biotecnología CNB-CSIC, Microbial Biotechnology, Madrid, Spain*

Backgrounds

FtsZ, a cytoplasmic GTPase, is the major component of the ring-shaped cell division machinery (the divisome) of most bacteria. Its polymerization plays an important role in constriction. In *Escherichia coli*, alteration of the FtsZ lateral contacts modifies its behaviour *in vitro* and *in vivo*.

The FtsZ·E93C mutant, retaining 20% of GTPase activity, can partially rescue VIP2, a conditional FtsZ strain (Encinar *et al.,* 2013). Mutations in the nearby residue E38 (at 8.5 Å distance) showed abnormal pole morphology and twisted septa (Bi and Lutkenhaus, 1992).

Objectives

Analysis of the *in vivo* effects of the FtsZ E38 and E93 lateral area. Comparison with mutations D269A and D86K (Stricker and Erickson, 2003), having a lower GTPase activity but located at different sides than E38 and E93.

Methods

Scanning and transmission electron microscopy were used to visualize the morphology of *E. coli* VIP2 derivatives in which FtsZ$^+$ is replaced by point mutants in E38 and E93. Growth, morphological parameters and localization of division proteins were measured.

Conclusions

We found a negatively charged area of *E. coli* FtsZ comprising E38 and E93 involved in divisome disassembly but not in its formation. This evident “FtsZ disassembly phenotype” shows detachment of the envelope layers and persistence of abnormally wide Z-rings both at midcell and at cell poles. It suggests a unique role of this lateral side, differently from those containing D269A or D86K. Mutations in E38E93 showed reduced GDP/GTP exchange and low turnover in the divisome, likely caused by strong interactions among FtsZ protofilaments.

FEMS7-1907
Physiology / Biochemistry / Molecular Microbiology - Part III

SYMBIOTIC PLANT DECOMPOSITION BY FUNGI AND GUT MICROBES ASSOCIATED WITH FUNGUS-GROWING TERMITES

R. Rodrigues da Costa[1], K. Sydow Krogh Pedersen[1], H. Hu[1], J. Schückel[2], S. Kresimir Kracun[2], S. Vreeburg[3], B. Pilgaard[4], L. Lange[4], J. Harholt[5], D.K. Aanen[3], P. Sapountzis[1], M. Poulsen[1]
[1]University of Copenhagen, Department of Biology, Copenhagen, Denmark
[2]University of Copenhagen, Department of Plant and Environmental Sciences, Copenhagen, Denmark
[3]Wageningen University, Laboratory of Genetics, Wageningen, The Netherlands
[4]Technical University of Denmark, Department of Chemical and Biochemical Engineering-, Lyngby, Denmark
[5]Carlsberg A/S- Group Research, Carlsberg Research Laboratory, Copenhagen, Denmark

Backgrounds

Fungus-growing termites are a paramount example of symbiotic association between insect hosts, a saprotrophic fungal cultivar (genus *Termitomyces*), and co-diversified gut microbial communities. Through an intricate and efficient decomposition process that involves plant biomass passing termite guts before decomposition in the external fungal gardens, these termites fully utilize a range of plant substrates and have become major decomposers in the Old World.

Objectives

Previous studies have proposed complementarity in enzyme provisioning between the partners in the symbiosis, but targeted expression analyses have been lacking. Here we characterise plant polymer decomposition and the expression of enzymes that are produced during the decomposition process in three fungus-growing termite species from two genera.

Methods

We performed plant polymer content analyses in forage substrate and fungal gardens, enzyme assays of fungal garden and termite guts, and RNAseq and subsequent carbohydrate-active enzyme characterisation of fungal enzymes within gardens.

Conclusions

We found a remarkable decrease in the amount of plant polymers from the start (forage substrate) to the end of the decomposition process (old worker guts). This supports the capacity of the tripartite symbiosis to utilize nearly any complex plant polymer. Enzyme assays and RNAseq analyses supported these findings, as a wide range of plant-degrading enzymes was identified. The enzyme assays further suggested that termite ingestion of fungal nodules from the mature fungal gardens leads to the transport of enzymes to the freshly inoculated parts of the garden. This process likely boosts plant degradation in parts of the fungus garden where *Termitomyces* is not yet fully grown.

FEMS7-1701
Physiology / Biochemistry / Molecular Microbiology - Part III

PLASMID-MEDIATED HETEROZYGOSIS INCREASES PHENOTYPIC PLASTICITY IN BACTERIA

J. Rodríguez Beltrán[1], J. De la Fuente[1], J.C.R. Hernández[2], J.A. Escudero[3], R. Peña-Miller[2], C. MacLean[3], A. San Millán[1]
[1]IRYCIS. Ramon y Cajal Institute for Health Research, Plasmid Biology and Evolution lab, Madrid, Spain
[2]Center for Genomic Sciences UNAM, Microbial Systems Biology, Morelos, Mexico
[3]Oxford University, Department of Zoology, Oxford, United Kingdom

Backgrounds

Small multicopy plasmids are very frequent in prokaryotes. Their multicopy nature produces an increase in gene dosage for the resident loci that increases gene expression and the chances of acquiring beneficial mutations. However, once a mutation arises its fixation will be largely influenced, not only by its selection coefficient, but by the plasmid copy number, segregation and replication dynamics. Accordingly, plasmids carrying mutated genes will co-exist in the same cell with plasmids encoding the ancestral allele, under plasmid-mediated heterozygosis.

Objectives

In this work we aim to explore the consequences of plasmid-mediated heterozygosis. It is known that when proteins acquire a new function through mutation they usually lose effectiveness at their original activity. We predict that plasmid-mediated heterozygosis may alleviate these trade-off effects that occur during evolutionary innovation.

Methods

To test this hypothesis, we combined mathematical modeling with an experimental system using *Escherichia coli* and fluorescently-labeled multicopy plasmids carrying either TEM-1 or TEM-12 β-lactamases. TEM-1 and TEM-12 are separated by a single mutation and confer resistance to ampicillin and ceftazidime respectively, showing a good example of an evolutionary trade-off.

Conclusions

We show that strains carrying both TEM alleles under heterozygosis are able to withstand concentrations of ceftazidime and ampicillin, alone or in combination, better than any of their homozygotic counterparts. Moreover, plasmid-mediated heterozygosis confers phenotypic plasticity to bacterial populations, facilitating the adaption to a range of constant and fluctuating antibiotic selection regimes. We argue that multicopy plasmids alleviate evolutionary trade-offs by maintaining copies of ancestral and evolved alleles under heterozygosis.

FEMS7-1309
Physiology / Biochemistry / Molecular Microbiology - Part III

HEME UPTAKE SYSTEMATIC FUNCTIONAL ANALYSIS IN NONTYPABLE HAEMOPHILUS INFLUENZAE IDENTIFIES A NOVEL FAMILY OF HEME BINDING-DONATION MOONLIGHTING PROTEINS

I. Rodriguez-Arce[1], F.C. Ariadna[1], A.J. Tamim[2], E. Begoña[3], M. Javier[1], C. Lucia[1], M. Sara[3], R. Kristian[4], G. Juncal[1,3]
[1]Instituto de Agrobiotecnología-CSIC, Animal Healthcare, Pamplona, Spain
[2]Lund University, Clinical Microbiology- Department of Translational Medicine, Malmö, Sweden
[3]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias, CIBERES, Madrid, Spain
[4]Lund University, Clinical Microbiology- Department of Translational Medicine, Malmo, Sweden

Backgrounds

The respiratory pathogen nontypeable *Haemophilus influenzae* (NTHi) is an important cause of exacerbations in patients suffering from chronic obstructive pulmonary disease (COPD), a progressive disease involving airway restriction, alveolar destruction and loss of lung function, primarily due to cigarette smoke (CS) exposure. CS induces the cytoprotective enzyme heme oxygenase-1, which catalyzes heme degradation. The existence of multiple heme-uptake systems in NTHi may be a evolutionary adaptation to its naturally-acquired heme auxotrophy, and also to overcoming host nutritional immunity and heme limited availability in the airway. Moreover, several NTHi heme-uptake systems are multifunctional proteins involved not only in heme capture, but also in bacterial resistance to antimicrobial peptides or epithelial adhesion. Relative contribution of the repertoire of NTHi heme-uptake systems to its host interplay is currently unknown.

Objectives

Functional characterization of a panel of 8 NTHi heme-uptake systems and their contribution to this host-pathogen interplay.

Methods

NTHi mutants lacking the TonB, HgpB, HxuCBA, TbpAB SapABCDZ, HitABC, HbpA-DppBCDF or protein E iron/heme/haemoglobin/haemopexin-capture systems were generated, and characterized in terms of bacterial growth, heme-binding and inter-bacterial donation, antimicrobial resistance, resistance to oxidative stress, relative gene expression, bacterial interplay with airway epithelia and *in vivo* lung infection.

Conclusions

NTHi heme-uptake systems are not hierarchically organized neither gene expression- nor function-wise. SapA, HxuA, DppA and protein E, besides other functions, share their ability to bind and donate heme, therefore constituting a novel family of moonlighting proteins.

FEMS7-1825
Physiology / Biochemistry / Molecular Microbiology - Part III

DESIGN OF A REAL-TIME PCR TOOL TO STUDY CELL WALL STRESS IN FUNGI

L. da Cruz Cabral[1], J. Delgado[1], M.J. Andrade[1], A. Patriarca[2], M.A. Asensio[1], A. Rodríguez[1]
[1]Meat and Meat Products Research Institute, Animal Production and Food science, Cáceres, Spain
[2]Buenos Aires University, Organic Chemistry, Buenos Aires, Argentina

Backgrounds

The cell wall integrity (CWI) pathway is responsible for the reparation and/or biosynthesis of the cell wall and is activated when changes on the cell surface occurred mainly under imposed stress. A new tool able to detect changes in stress-related genes would be useful to understand the action mechanism of some filamentous fungi against antifungal compounds.

Objectives

The objective was to develop a real-time PCR (qPCR) using SYBR Green to monitor changes in the *Rho1* gene expression levels in moulds.

Methods

Optimization of reaction conditions included evaluation of different primer pairs, primer concentrations and annealing temperatures/times. The final reaction mixture contained 200nM of each primer and an annealing temperature of 55ºC. The specificity of primers was demonstrated when amplified a unique qPCR product with a Tm value of 86ºC. The qPCR showed a R^2 value =0.9994 and amplification efficiency of 97.5%. The method was validated by treating *Aspergillus flavus* and *Penicillium polonicum* with the antifungal protein PgAFP. The PgAFP-resistant *P. polonicum* showed an overexpression of *Rho1*, while the opposite trend was detected in *A. flavus* with the antifungal treatment.

Conclusions

This qPCR assay is a valuable tool to analyse intracellular responses linked to CWI pathway activation. This provides data to in-depth understand the ability of fungi to colonise different environments and to develop new antifungals

Acknowledgements: L. da Cruz Cabral is recipient of a pre-doctoral fellowship (BEC.ar call 2015). Dr. A. Rodríguez is supported by a Juan de la Cierva senior research fellowship (IJCI-2014-20666)

FEMS7-2302
Physiology / Biochemistry / Molecular Microbiology - Part III

PHOSPHORYLATION OF YEAST CTH2 PROTEIN AND ITS SUBSEQUENT DEGRADATION BY GRR1 ARE REQUIRED FOR OPTIMAL GROWTH IN IRON DEFICIENT CONDITIONS

A.M. Romero[1], M.M. Martínez-Pastor[2], N. Sanvisens[3], J.A. Wohlschlegel[4], D. Toczyski[3], M.T. Martínez-Pastor[5], D.J. Thiele[2], S. Puig[1], S. Vergara[6]
[1]CSIC-IATA, Biotechnology, Paterna, Spain
[2]Duke University School of Medicine, Pharmacology and Cancer Biology, Durham- North Carolina, USA
[3]Helen Diller Family Comprehensive Cancer Center, Biochemistry and Biophysics, San Francisco- California, USA
[4]University of California, Biological Chemistry, Los Angeles- California, USA
[5]University of Valencia, Biochemistry and Molecular Biology, Burjassot- Valencia, Spain
[6]Duke University, Biology, Duke, USA

Backgrounds

Iron is an essential micronutrient for most organisms because it participates as a redox cofactor in a variety of metabolic pathways. However, iron bioavailability for living organisms is highly restricted due to its low solubility at physiological pH. Upon iron scarcity, *Saccharomyces cerevisiae* Cth2 protein, characterized by the presence of two $Cx_8Cx_5Cx_3Hx_{18}Cx_8Cx_5Cx_3H$ tandem zinc fingers (TZFs), is expressed and binds to AU-rich elements (AREs) of many mRNAs encoding for iron-containing proteins, promoting their degradation. Thus, Cth2 optimizes iron utilization by repressing non-essential iron-consuming pathways (respiration) and activating indispensable iron-dependent processes (DNA synthesis). Yeast cells need to fine-tune the expression levels of Cth2 protein because its excess can be detrimental for growth. Previous studies have demonstrated that Cth2 limits its expression by binding to AREs within its own mRNA, promoting its auto-degradation.

Objectives

Our goal is to characterize the post-translational mechanisms that regulate Cth2 protein levels according to environmental iron bioavailability. Cth2 phosphorylation, ubiquitylation and degradation by the proteasome will be studied.

Methods

RNA (qPCR) and protein (Western blot, CoIP) analyses, yeast growth assays

Conclusions

Yeast Cth2 protein is phosphorylated at serine residues 65, 68 and 70 under iron-deficient conditions. Mutagenesis of these serine residues does not eliminate Cth2 targeted mRNA degradation function, but it increases Cth2 protein stability. Our data demonstrate that F-box E3-ligase protein Grr1 recognizes phosphorylated Cth2 and facilitates its degradation by the proteasome. Both mutagenesis of Cth2 serine residues and *GRR1* deletion cause significant growth defects under iron depletion, emphasizing the physiological relevance of regulating Cth2 protein levels.

FEMS7-1666
Physiology / Biochemistry / Molecular Microbiology - Part III

PSEUDOMONAS AERUGINOSA IN CYSTIC FIBROSIS CHRONIC INFECTIONS: HOW THE AIRWAY ENVIRONMENT SHAPES THE BACTERIAL TRANSCRIPTOME

E. Rossi[1], S. Molin[2], H.K. Johansen[1]
[1]Rigshospitalet, Department of Clinical Microbiology, Copenhagen Ø, Denmark
[2]Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Kgs. Lyngby, Denmark

Backgrounds

Cystic fibrosis (CF) is a life-threatening disease associated with genetic mutations affecting the activity of the epithelial chloride transporter CFTR. This condition promotes the formation of a viscous mucus layer that covers epithelial tissues of airways, creating an optimal environment for bacterial growth and development of chronic infections. *P. aeruginosa* is a predominant pathogen in these infections, mostly due to its ability to survive and evolve in CF lungs. Although progress has been made in understanding *P. aeruginosa* adaptive strategies, little is known about its behaviour in human lungs. In particular, we known very little about the ways *P. aeruginosa* physiology is shaped by the unique chemico-biological environment of CF patients' airways.

Objectives

Using a meta-transcriptomics approach, we investigate the eco-physiology of *P. aeruginosa* communities directly in human CF sputum.

Methods

CF sputum is collected and the DNA/RNA content stabilized within less than a minute. After rRNA depletion, isolated RNA is used for the preparation of sequencing libraries. High-depth sequencing is performed on Illumina NextSeq platform. *In silico* analysis is carried out using a customized pipe-line.

Conclusions

Our preliminary results picture a highly dynamic transcriptionally-active microbial community in CF patients. From our high-resolution approach, we could quantitatively evaluate *P. aeruginosa* gene expression in the patient's lungs, disclosing a peculiar expression profile characterized by functions that reflect bacterial adaptation to CF airways (anoxia, metal ion limitation, oxidative and antibiotic stresses), and features typical for both fast-growing and slow-growing cells, underlining the evolved physiology of the bacterium at the stage of long-term chronic infections.

FEMS7-2592
Physiology / Biochemistry / Molecular Microbiology - Part III

EVOLUTIONARY INFORMATION VALUE OF GENOMIC ISLANDS OF ROOT NODULE BACTERIA SINORHIZOBIUM SPP.

M. Roumiantseva[1], V. Muntyan[1], M. Cherkasova[1], A. Saksaganskaya[1], B. Simarov[1]
[1]ARRIAM, Laboratory of Genetics, Saint Petersburg Pushkin, Russia

Backgrounds

Genomic islands (GIs) are not the typical accessory elements of the bacterial genome. The spectrum of GIs is represented by classical types like “pathogenicity”(PAIs) or "symbiotic” (SI) islands up to islands inheriting an extent blocks of genes with unknown functions. GIs are site specific integrated units in sequences of tRNA or tmRNA. Typically they have direct repeats, phage integrase, abundant IS-elements, transposons, noncoding RNA (ncRNA), as well as a numerous "foreign" genes, repetitive sequences, plasmid DNA sequences of various species of microorganisms. Genes inherited by GIs can dramatically improve and expand bacteria habitats (fitness).

Objectives

We are going to present and discuss evolutionary information value of genomic islands in *Sinorhizobium spp*. and GIs distribution in *S. meliloti* populations native to geographically distant centers of alfalfa diversity impact by different abiotic stress factors.

Methods

New molecular genetic approaches in tight links with bioinformatics and synthetic methods are providing tremendous comparative evaluation of *Sinorhizobium spp*. genomes.

Conclusions

Chromosome of *S. meliloti* 2011 comprises at least three elongated areas that are considered as genomic islands. These regions were not previously investigated, but it is known that they contain at least 196 ORF, IS-elements 5 identified families and 6 unidentified types and genes responsible for at least 2 types of restriction-modification systems and 75 ncRNA. The geographical distribution of genomic islands in *S. meliloti* populations and thier evolutionary information value in *Sinorhizobium spp*. will be discussed.

FEMS7-2510
Physiology / Biochemistry / Molecular Microbiology - Part III

IDENTIFICATION OF A UNIQUE GENETIC SIGNATURE IN A NOVEL COMPLEX RESISTANCE GENE LOCUS IN MULTIPLE DRUG RESISTANT UROPATHOGENIC ESCHERICHIA COLI ST405

P. Roy Chowdhury[1], *J. McKinnon*[1], *M. Liu*[1], *S. Djordjevic*[1]
[1]*University of Technology Sydney, The ithree institute- Faculty of Science, Sydney, Australia*

Backgrounds

Escherichia coli belonging to sequence type (ST) 405 is globally known for dissemination of $bla_{CTX\text{-}M15}$ and NDM-1 genes and is noted threat to human health. In the course of our routine surveillance of multiple drug resistant (MDR) *E. coli* strains circulating in hospitals in Sydney, Australia, we identified *E. coli* isolate 2009-27 from midstream urine of a patient with cystitis that was resistant to most first line antibiotics, including ampicillin, azithromycin, kanamycin, sulphafurazole and trimethoprim used in the treatment of urinary tract infections. It was however sensitive to extended spectrum betalactams. Our in-house targeted PCR screening data indicated that 2009-27 contained a complex resistance locus (CRL), which could serve as a hotspot for further accumulation of resistance genes.

Objectives

To characterize the CRL in MDR strain 2009-27 and identify genetic signatures to reliably track dissemination of this clonal ST405 lineage.

Methods

Strain 2009-27 was sequenced using PacBio technology and interrogated using online analysis tools including BLAST, ORF finder, RAST, eMLST and ISfinder.

Conclusions

Strain 2009-27 was identified as phylogenetic group B2, ST405 with serotype O102:H6. A laterally-acquired, chromosomally-located complex resistance gene locus, spanning almost 20 kb, was shown to contain a suite of resistance genes that was sufficient to account for the resistance phenotype of strain 2009-27. The CRL also included five copies of the insertion element IS*26*. IS*26* appears to have played a major role in the evolution of the CRL in 2009-27, including creation of a unique deletion which can be exploited to track this MDR lineage.

FEMS7-2884
Physiology / Biochemistry / Molecular Microbiology - Part III

THE PANGENOME OF AN UNCULTURED NOVEL BACTEROIDETES SPECIES

M. Royo-Llonch[1], P. Sánchez[1], J.M. González[2], C. Pedrós-Alió[3], S.G. Acinas[1]
[1]Institut de Ciències del Mar- CSIC, Department of Marine Biology and Oceanography, Barcelona, Spain
[2]Universidad de La Laguna, Department of Microbiology, San Cristóbal de la Laguna, Spain
[3]Centro Nacional de Biotecnología- CSIC, Systems Biology Department, Madrid, Spain

Backgrounds

The uncertainty on the threshold that defines bacterial species induced the “Pan-genome” concept. It categorizes prokaryotic species as a group of genomes that share a "core genome" and differ in the “flexible pool genome”. The first one codes for central metabolism and highly conserved genes, while the second is the sum of dispensable genes of each genome’s taxa. The flexible pool varies within the species due horizontal gene transfer and other genome rearranging events. Traditionally, pangenomes have been produced with isolated bacteria. Nowadays, retrieval of diverse genomes from natural samples by Single Cell Genomics (SCG) allows the exploration of the pangenome of those abundant microbial genomes that elude cultivation.

Objectives

The aim of our research is to generate the Pangenome of a novel photoheterotrophic *Kordia* species (Bacteroidetes) through environmental genomes obtained by SCG.

Methods

We retrieved 98 apparently clonal *Kordia* Single Amplified Genomes (SAGs) from the Indian Ocean, 10 of which have been sequenced. Reconstruction of most of this *Kordia* sp. genome has been possible through a co-assembly. Their genomic comparison, together with two phylogenetically identical SAGs retrieved from the North Pacific Subtropical Gyre deep waters will help us to elude the size of this *Kordia* sp. core and flexible genomes, which processes may have generated the flexible genome, differences in genetic composition of each flexible genome, etc.

Conclusions

In conclusion, this work features two significant points in marine bacterial genomics: firstly, the assembly and functional annotation of 10 novel Bacteroidetes SAGs and secondly, the construction and analysis of this *Kordia* sp.’ Pangenome.

FEMS7-2781
Physiology / Biochemistry / Molecular Microbiology - Part III

FOOTPRINTS OF POSITIVE NATURAL SELECTION IN PNEUMOCYSTIS SPECIES

L. Delaye[1], S. Ruiz-Ruiz[2], E. Calderón[3], A. Conesa[4], A. Moya[2]
[1]Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional CINVESTAV, Departamento de Ingeniería Genética, Irapuato, Mexico
[2]Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comuni, Genómica y Salud, Valencia, Spain
[3]CIBER de Epidemiología y Salud Pública- Instituto de Biomedicina de Sevilla- Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Departamento de Epidemiología, Sevilla, Spain
[4]Centro de Investigación Príncipe Felipe, Genomics of Gene Expression, Valencia, Spain

Backgrounds

Background

Pneumocystis species are ascomycete fungi that adapted to live inside the lungs of mammals. These ascomycetes show extensive stenoxenism, such that each *Pneumocystis* species can infect only a single host species, indicating a high level of adaptation to and co-evolution with its host.

Objectives

Objectives

The aim of this project is to study the pattern exerted by natural selection on functionally related sets of genes in the genome of *Pneumocystis carinii* (rats), *Pneumocystis murina* (mice) and *Pneumocystis jirovecii* (humans). In addition, we studied the extent of positive selection and recombination in the evolution of major surface glycoproteins (Msg).

Methods

Methods

Protein families were aligned with MUSCLE and for each gene family, we estimated synonymous (dS) and non-synonymous (dN) rates of substitution. To detect recombination in Msg proteins we used GARD from HyPhy 2.220 package and the episodic diversifying selection was detected with MEME on each of the recombinant segments identified with GARD.

Conclusions

Conclusions

We showed that genes with an increased rate of non-synonymous substitution compared to the rest of genes in the genome coding for glycosylphosphatidylinositol biosynthesis in *Pneumocystis jirovecii* and *Pneumocystis carinii*, and for complexes of the protein secretion apparatus in *Pneumocystis jirovecii*. Also, we found that between 12% to 30% of the sites in Msg proteins evolved by episodic positive selection and identified several recombination segments along coding genes. These data suggest strong selective pressure of the immune system of the host in shaping the evolution of Msg proteins and the cell machinery implicated in protein secretion in *Pneumocystis*.

FEMS7-2040
Physiology / Biochemistry / Molecular Microbiology - Part III

THE PHENOTYPIC PLASTICITY OF DUPLICATED GENES IN SACCHAROMYCES CEREVISIAE AND THE ORIGIN OF ADAPTATIONS

B. Sabater-Munoz[1,2,3], F. Mattenberger[1,2], C. Toft[4,5], M.A. Fares[1,2,3]
[1]Instituto de Biologia Molecular y Celular de Plantas IBMCP del CSIC UPV, Abiotic Stress, Valencia, Spain
[2]Institute for Integrative Systems Biology I2SysBio from CSIC,
Systems biology of molecular interactions and regulation, Valencia, Spain
[3]Smurfit Institute of Genetics- Trinity College of Dublin, Genetics, Dublin, Ireland
[4]University of Valencia UV, Genetics, Valencia, Spain
[5]Instituto de Agroquimica y Tecnología de los Alimentos IATA - CSIC, Biotechnology, Valencia, Spain

Backgrounds

Gene and genome duplication are major sources of biological innovations in all kingdoms, including yeast, plants and animals. As an example, a whole-genome duplication event provided the genomic background for the evolution of an efficient anaerobic fermentation capacity and the respiro-fermentative life-style in Saccharomyces yeasts (budding yeasts). Functional and transcriptional divergence between the copies after gene duplication has been considered the main driver of innovations. However, the relative role of transcriptional evolution of duplicated genes in the origin of adaptations remains poorly characterized.

Objectives

Here, we study the phenotypic plasticity of duplicated genes and determine its role in the origin of adaptations in *S. cerevisiae*.

Methods

The unicellular eukaryote *Saccharomyces cerevisiae* Y06240 haploid strain was challenged with five different environmental stresses (oxidative, ethanol, glycerol, lactate and oxidative plus dextrose). Transcriptional alterations were determined by RNAseq and differential expression analyses. The transcriptional plasticity of singletons and duplicates was analyzed. We also analyzed the role of the mechanism of duplication (whole-genome vs small-scale) in the transcriptional plasticity of duplicates.

Conclusions

The transcriptional response of *S. cerevisiae* to environmental stresses was greater through the alteration of duplicates than singletons. Duplicated genes exhibited signatures of adaptive transcriptional patterns in response to stress. Whole genome duplicates were more transcriptionally altered than small-scale duplicates. Duplicates responded more specifically to stress than singletons, supporting the role of natural selection in the transcriptional plasticity of duplicates. Our results reveal the underlying transcriptional complexity of duplicated genes and its role in the origin of adaptations.

FEMS7-2062
Physiology / Biochemistry / Molecular Microbiology - Part III

GROE OVEREXPRESSION, ENDOSYMBIOSIS AND MUTATIONAL ROBUSTNESS

B. Sabater-Munoz[1,2,3], *R. Montagud-Martinez*[1,3], *C. Toft*[4,5], *M.A. Fares*[1,2,3]
[1]*Instituto de Biologia Molecular y Celular de Plantas IBMCP del CSIC - UPV, Abiotic Stress, Valencia, Spain*
[2]*Smurfit Institute of Genetics- University of Dublin- Trinity College, Genetics, Dublin, Ireland*
[3]*Institute for Integrative Systems Biology I2SysBio CSIC-UV,*
Systems Biology of Molecular Interactions and Regulation, Valencia, Spain
[4]*University of Valencia, Genetics, Valencia, Spain*
[5]*Instituto de Agroquímica y Tecnología de los Alimentos IATA CSIC, Biotechnology, Valencia, Spain*

Backgrounds

The populations of endosymbiotic bacteria of insects are subjected to strong bottlenecks during their transmission to the host offspring, making selection less efficient on purging deleterious mutations. General endosymbiotic symptoms include reduced bacterial genome sizes, novel biochemical adaptations and increased genome mutational load. Despite these detrimental evolutionary dynamics, endosymbiotic bacteria of insects have been preserved through millennia. The underpinnings of the successful endosymbiotic lifestyle remain unclear. Some evidence point, however, to a fundamental role of the molecular chaperonin GroEL in bufferring the effects of deleterious mutations and enabling the origin of adaptations in endosymbiotic bacteria.

Objectives

This work aims to determine the role of the chaperonin GroEL in the phenotypic evolution through conferring resistance to deleterious mutations in bacterial populations evolving under genetic drift.

Methods

Escherichia coli (MG1655 delta *mutS*) bacterial isogenic lines over-expressing the operon *groE* were subjected to 180 (~4000 generations) daily passages under strong genetic drift effects, resembling matrilines transmissions of endosymbiotic bacteria of insects. The genomes of the evolved populations were sequenced using Illumina technology. Mutations arising during the evolution experiments were determined by comparing the genomes of evolved populations with those of their ancestral population. The effects of the mutations were estimated by calculating the fitness of the evolved populations relative to their ancestral population.

Conclusions

GroE over-expression confers resistance to deleterious mutations. The return of *groE* to its wild type expression levels led to a decline in the fitness and increase in the number of aberrant phenotypes in the evolved population.

FEMS7-2047
Physiology / Biochemistry / Molecular Microbiology - Part III

EVOLUTION OF STAPHYLOCOCCUS AUREUS IN A MURINE MODEL OF NASOPHARYNGEAL COLONISATION

B. Salgado[1], J. Moran[1], E. Waters[2], A. Kadiolgu[2], M. Horsburgh[1]
[1]University of Liverpool, Institute of Integrative Biology, Liverpool, United Kingdom
[2]University of Liverpool, Institute of Infection and Global Health, Liverpool, United Kingdom

Backgrounds

Staphylococcus aureus is a frequent member of the human nasopharyngeal microbiome. The features that contribute to colonisation and the selective pressures in the presence of competitor species aren't clear.

Objectives

To better understand how *S. aureus* both colonises and persists in the nasopharynx, we developed a long-term asymptomatic model of nasopharyngeal colonisation in mice.

Methods

A nasopharyngeal colonisation model was established using CD-1 mice with *S. aureus* USA300 LAC*. To examine the selective pressure of this infection model upon *S. aureus,* serial passages were performed over 7 days. Three separate consecutive passages were performed with *S. aureus* USA300 LAC* being isolated between passages and used to establish a repeat colonisation with identical load. Following each passage 10 *S. aureus* isolates were obtained and their genomes were purified, pooled and genome resequenced. A single clone from the second passage was randomly picked and sequenced. The genome sequences of each pool of 10-strains and the evolved isolate were compared with their isogenic parent to identify the identity and frequency of SNPs/indels. Complementary *in vivo* assays were performed to assess phenotype changes by the evolved strain.

Conclusions

Bioinformatics analysis revealed distinct patterns of genetic changes after each subsequent cycle of *S. aureus* colonisation. Sequencing revealed incremental numbers of SNPs/indels with successive passages. After three rounds of colonisation there was evidence of repeated selection at specific loci. The observed selection pressure outcomes were mapped to cellular pathways to identify *in vivo* adaptation. A clone from the second passage showed increased colonisation frequency relative to its isogenic parent.

FEMS7-2580
Physiology / Biochemistry / Molecular Microbiology - Part III

TRANSCRIPTOME ANALYSIS OF TELOMERASE NEGATIVE STRAINS OF USTILAGO MAYDIS

M.P. Sánchez-Alonso[1], J.J. Jacinto Vázquez[2], E. Anastacio Marcelino[3], C. Vázquez Cruz[3]
[1]Instituto de Ciencias, Centro de Investigaciones en Ciencias Microbiológicas, Puebla, Mexico
[2]Instituto de Ciencias- Benemérita Universidad Autónoma de Puebla,
Posgrado en Microbiología- Centro de Investigaciones en Ciencias Microbiológicas, Puebla, Mexico
[3]Instituo de Ciencias- Benemérita Universidad Autónoma de Puebla,
Centro de Investigaciones en Ciencias Microbiológicas, Puebla, Mexico

Backgrounds

The *U. maydis trt1::hph* mutants lacking telomerase activity exhibit low replicative potential, delayed growth, short telomeres, and senescent traits in same way as *est2* mutants in yeasts. As in *est2* cells, survivors sporadically arise among telomerase-negative mutants that maintain telomere function by recombination-based mechanisms known as alternative lengthening of telomere (ALT). Molecular machinery of RNA metabolism is, at least in part, involved in these arising of survivors.

Objectives

In this work we initiate transcriptome analysis of telomerase-negative Type I-like and Type II-like survivor mutants of *U. maydis* in order to compare their global transcription profiles with that of the parental strain 521.

Methods

High-quality total RNA from all the three strains was extracted, and sent to LANGEBIO-CINVESTAV Campus Irapuato to be processed and sequenced in order to obtain poly (A) transcriptomes. Sequencing was performed by duplicate using the Ion-Torrent NGS platform of Life Sciences. Assemble was made using Trinity software (https://github.com/trinityrnaseq/trinityrnaseq/wiki).

Conclusions

Raw analysis render 73,623 potential transcripts from transcriptomes of 521 strain and from Type I mutants in independent experiments by duplicate; length of transcripts range from 201 to 4010 bp with a total of assembled bases of 40,304,870 and N50 = 752 bp, In similar analysis we found 70,828 potential transcripts form parental strain and Type II mutant from two independent replicates; here were assembled 29,396,441 bp. Length of transcripts ranged form 201 pb to 4254, N50 = 437. Preliminary differential expression analyses showed changes in gene expression in Type I and Type II ALT mutants respect to wild type strain.

FEMS7-0223
Physiology / Biochemistry / Molecular Microbiology - Part III

EFFECT OF THE HFQ AND CRC PROTEINS ON THE TRANSCRIPTOME OF PSEUDOMONAS PUTIDA

D.L. Sánchez-Hevia[1], *R. Moreno*[1], *F. Rojo*[1]
[1]*Centro Nacional de Biotecnología, Department of Microbial Biotechnology, Madrid, Spain*

Backgrounds

In metabolically versatile bacteria such as *Pseudomonas putida*, catabolite repression facilitates the preferential assimilation of the most efficient carbon sources, optimizing metabolism and improving growth rate and fitness. In *P. putida*, catabolite repression is mediated by the Hfq and Crc proteins, which inhibit translation of target mRNAs. Two sRNAs, CrcZ and CrcY, can bind these proteins and antagonize their activity. Inactivation of the gene coding for Hfq leads to a much stronger phenotype than inactivation of *crc*, suggesting that while Crc needs Hfq, Hfq might regulate some genes independently of Crc. In other bacterial species in which Crc is not present, Hfq is involved in many post-transcriptional regulation processes.

Objectives

Our aim was to elucidate whether Hfq can regulate genes in the absence of Crc, and vice-versa, and identify those genes.

Methods

Previous studies have indicated that although Crc and Hfq are post-transcriptional regulators, their absence has a great effect on the cell transcriptome. Therefore, the transcriptome of wild type, Hfq-null, Crc-null, and Hfq/Crc-null strains cultivated in a mineral salts medium with succinate as carbon source was analysed using RNA-Seq.

Conclusions

The absence of Hfq had a much greater effect on the transcriptome than the absence of Crc. Genes regulated by Crc were also Hfq-dependent. However, many genes were regulated by Hfq but not by Crc. Among others, this includes the genes involved in iron homeostasis. Current understanding on the underlying reasons will be presented.

FEMS7-1955
Physiology / Biochemistry / Molecular Microbiology - Part III

OSMOREGULATORY MECHANISMS OF THE SUCCESSFUL HALOPHILIC BACTERIA SPIRIBACTER

M.J. Leon[1], T. Hoffman[2], C. Sánchez-Porro[1], E. Bremer[3], A. Ventosa[1]
[1]Universidad de Sevilla, Microbiology and Parasitology, SEVILLA, Spain
[2]Philipps-University Marburg, Department of Biology-, Marburg, Germany
[3]Philipps-University Marburg, Department of Biology, Marburg, Germany

Backgrounds

Studies carried out in hypersaline environments focused on culture-independent techniques showed that microorganisms easily isolated in the laboratory do not constitute the predominant microbiota and therefore they are not the ideal models for understanding the relationships and functions of microbes in natural environments. Metagenomic-based studies in salterns have recently permitted the isolation of representatives of a dominant bacterial group at intermediate salinities (10-20 % salts) that have been named as a new genus, *Spiribacter.*

Objectives

We have combined a physiological approach and genome mining to derive a comprehensive picture of the molecular and cellular events that allow to the members of the genus *Spiribacter* to be successful bacteria in hypersaline environments.

Methods

We have used a qualitative and quantitative assessment by natural abundance ^{13}C-NMR spectroscopy and high performance liquid chromatography analysis, respectively, and a genomic analysis of the species of this genus.

Conclusions

The genus *Spiribacter* represents the first ecologically defined moderately halophilic bacteria, quite abundant at intermediate salinities. Our data show that newly synthesized ectoine and imported glycine betaine play key roles in the osmoregulation of *Spiribacter salinus* to the high salinity. Species of *Spiribacter* and taxonomically related species possess an unusual genetic organization of ectoine biosynthetic genes and notably they lacked the gene *ectD* for the ectoine hydroxylase. Experimental results showed that *Spiribacter* species are able to synthesize the compatible solute ectoine, showing a linear relationship between the synthesis of this compatible solute and the osmotic stress. *Spiribacter salinus* was also able to uptake glycine betaine from the environment and use it as a carbon and energy source.

FEMS7-2635
Physiology / Biochemistry / Molecular Microbiology - Part III

INTEGRATING PROTEOMIC RESEARCH INTO DIFFERENT BACTERIAL SPECIES WITH GENOMIC DATA AND BIOINFORMATICS TOOLS

T. Santos[1,2], C. Marinho[1,2], P. Poeta[3,4], G. Igrejas[1,2,4]
[1]University of Trás-os-Montes and Alto Douro, Department of Genetics and Biotechnology, Vila Real, Portugal
[2]University of Trás-os-Montes and Alto Douro, Functional Genomics and Proteomics Unit, Vila Real, Portugal
[3]University of Trás-os-Montes and Alto Douro, Veterinary Science Department, Vila Real, Portugal
[4]Faculty of Science and Technology- University Nova of Lisbon, UCIBIO-REQUIMTE, Lisbon, Portugal

Backgrounds

Proteomes are dynamic, varying according to cell type, functional state and responses to stress, such as sensitivity or resistance to antibiotics. Antimicrobial resistance presents a challenge to scientists in the field of infectious diseases. The full knowledge of how antibiotic resistance is evolving and being transmitted between hosts in different ecosystems is taking on great importance. The Functional Genomics and Proteomics Unit has recently completed 10 years of proteomics research related to antimicrobial resistance. The evaluation of protein profiles related to antimicrobial resistance is a valid approach in the development of new therapeutic strategies.

Objectives

To clarify antibiotic resistance mechanisms of multiresistant bacterial strains by integrating genomics and proteomics in a multidisciplinary framework.

Methods

Five different bacterial species were studied by isolating 32 strains from clinical and wildlife samples. A total of 2770 protein spots were characterized through 2-DE and MALDI-TOF MS, and 392 proteins were identified by shotgun proteomics (LC-MS/MS).

Conclusions

Our group has evaluated ESBL-producing Escherichia coli strain protein profiles, compared proteomes of vancomycin-resistant Enterococcus spp. strains, analysed the whole proteome of quinolone-resistant Salmonella strains, analysed a sub-proteome of a methicillin-resistant Staphylococcus aureus strain and undertaken several other proteomic approaches. Our aim is to present the framework connecting this large protein database with other published results on antimicrobial resistance to elucidate the metabolic pathways that result in antimicrobial behaviour. The identification of protein determinants of resistance not only provides biomarkers for resistance to a particular drug, but also aids in the understanding of the mechanisms of antibiotic function and resistance.

FEMS7-1707
Physiology / Biochemistry / Molecular Microbiology - Part III

NEW INSIGHTS INTO THE ROLE OF FURC IN NITROGEN METABOLISM AND HETEROCYST DIFFERENTIATION

C. Sarasa[1], E. Sevilla[1], E. Broset[1], M.F. Fillat[1], M.L. Peleato[1]
[1]Faculty of Sciences and Biocomputation and Complex Systems Physics Institute BIFI- University of Z aragoza,
Department of Biochemistry and Molecular and Cell Biology- Biochemical- Biophysical and Computati onal Biology Group - CSIC Joint Unit, Zaragoza, Spain

Backgrounds

FUR (ferric uptake regulator) proteins constitute a large family of transcriptional regulators that exhibit a wide range of functions. FurC from *Anabaena* sp. PCC 7120 participates in the modulation of other FUR paralogues and is related to the response to oxidative stress. Furthermore, overexpression of the master regulator of nitrogen control in cyanobacteria NtcA leads to an increase of the transcription of FurC, suggesting its relationship to nitrogen metabolism. NtcA also regulates several genes, such as *nblA, a* gene responsible for the degradation of phycobilisomes, an early response under nitrogen starvation.

Objectives

To gain more insights on the role of FurC in nitrogen metabolism in *Anabaena* sp. including heterocyst differenciation.

Methods

Transcriptional analysis were performed using real-time PCR in the *Anabaena* sp. PCC 7120 wild type strain and the FurC-overexpressing mutant EB2770C, In order to determine a direct interaction between FurC and the promoter regions, DNA-protein binding assays (EMSA) were performed. Bright-field microscopy was used to determine the frecuency of heterocysts under nitrogen starvation.

Conclusions

Transcriptional analysis combined with EMSA indicated that FurC is either directly or indirectly involved in the regulation of several key genes such as *ntcA* and *nblA*. In addition, the *furC*-overexpressing mutant was unable to differenciate heterocysts under nitrogen deficiency. These results suggest the existence of a cross-regulation between NtcA and FurC and point to FurC as a transcriptional regulator involved in nitrogen metabolism in cyanobacteria.

FEMS7-1766
Physiology / Biochemistry / Molecular Microbiology - Part III

TRANSCRIPITONAL ANALISIS OF Γ-LINDANE INDUCED CHANGES IN MICROCYSTIS AERUGINOSA PCC7806 AND ANABAENA PCC7120

C. Sarasa[1], E. Sevilla[1], C. Castro[1], M.F. Fillat[1], M.L. Peleato[1]
[1]Faculty of Sciences and Biocomputation and Complex Systems Physics Institute BIFI- University of Z aragoza,
Department of Biochemistry and Molecular and Cell Biology- Biochemical- Biophysical and Computati onal Biology Group - CSIC Joint Unit, Zaragoza, Spain

Backgrounds

Cyanobacteria are organisms with an outstanding capacity to adapt and survive in extreme or highly degraded environments. Their metabolic plasticity includes the synthesis of a high level of potentially toxic secondary metabolites such cyanotoxins that cause serious health and environmental problems. Cyanobacteria are able to tolerate and even metabolize moderate doses of organochlorines pesticides, such as lindane (γ-hexachlorocychlohexane), one of the most persistent and used in recent decades. Previous work showed that *Microcystis aeruginosa* PCC7806 and *Anabaena* PCC7120 degrades lindane. While degradation routes of lindane are defined in *Sphingomonas*, in cyanobacteria remain unknown.

Objectives

Elucidate the genes involved in cyanobacteria in lindane degradative pathway, as well in its regulation.

Methods

In silico analysis of both *Microcystis aeruginosa* PCC7806 and *Anabaena* PCC7120 strains genomic sequences to identify homologies with the *lin* operon from *Sphingomonas.* Transcriptional analysis were performed using real-time PCR.

Conclusions

Transcripcional analysis of genes homologs of *lin* operon has been performed in *Microcystis* grown in the presence of lindane and in the *Anabaena furC*-overexpressing strain. Also, genes involved in the oxidative stress response and implicated in potential transcriptional regulation has been analysed. Among other changes, the presence of γ-lindane in the culture media induces *linE* expression as well as *prxA* gene transcription in *M. aeruginosa*. *Anabaena furC*-overexpression mutant showed a greater tolerance to γ-lindane, with a growth of approximately 90% respect to the control without lindane in all concentrations tested while the wildtype is about 75%. Transcriptional analysis of *lin* operon genes in *furC*-overexpression mutant will be presented.

FEMS7-1008
Physiology / Biochemistry / Molecular Microbiology - Part III

ADAPTATION OF B. MARMARENSIS SP NOV TO EXTREME ALKALINE CONDITIONS

F.E. Altinisik[1], D. Kazan[1], B. Sariyar Akbulut[1]
[1]Marmara University, Bioengineering, İstanbul, Turkey

Backgrounds

Bacillus marmarensis sp nov is an extreme obligate alkaliphile which can grow in a broad range of pH values between pH 8.0-12.5. Ability to grow at high pH values makes it a valuable microorganism since very few microorganisms can tolerate extreme alkaline conditions. This property may be used to by-pass sterilization processes in industrial applications, significantly lowering process costs.

Objectives

In the current study, the adaptation strategy of *B. marmarensis sp nov* to alkaline conditions was investigated using proteomic tools.

Methods

The organism was grown in nutrient broth (NB) medium. The final pH was adjusted to 9.8 or 10.6 with Na-sesquicarbonate solution. For extraction of total cellular proteins, cells were disrupted with MP Bio Fast Prep device. The proteome maps were obtained. Extracted proteins were treated protease inhibitor mix and nuclease mix. Then proteins were separated by 2-dimensional gel electrophoresis (2-DGE) using immobilized pH gradient (IPG) strips (17 cm and pH 3-6 gradient, Bio-Rad) in the first dimension and SDS-PAGE in the second dimension.

Conclusions

The obtained proteome maps showed significant changes in protein expression. Identification of the differentially expressed proteins will provide further clues on microbial alkaliphilic adaptation. This information could be used for manipulating other organisms for survival under alkaline conditions.

Funding Marmara University, Scientific Research Projects Committee (FEN-C-YLP-250816-0419) is gratefully acknowledged.

FEMS7-1132
Physiology / Biochemistry / Molecular Microbiology - Part III

STREPTOMYCES WERRAENSIS: A PROMISING CANDIDATE TO PRODUCE FERULOYL ESTERASE

A.K. Scheu[1], *J. Soliveri de Carranza*[1], *J.L. Copa-Patiño*[1]
[1]*Universidad de Alcalá, Biomedicina y Biotecnología, Alcalá de Henares, Spain*

Backgrounds

Feruloyl esterase (FAE) is an enzyme capable of hydrolysing the ester bond that links phenolic acids, such as ferulic acid (FA), to polysaccharides. In Nature, this enzyme plays a key role in plant cell wall biodegradation. FAE also finds application in many biotechnological processes in industry.

Objectives

Study of the production, purification, sequencing, and glucose repression of a FAE produced by *Streptomyces*.

Methods

The fermentation process has been optimized using a strain of *S. werranesis*. The FAE present in the culture broth has been purified and the gen sequenced using reverse genetics. Finally, glucose repression of the FAE encoding gene has been studied by transcriptional analysis.
In the present work, a new isolate of *S. werraensis* was selected for its FAE activity and the initial culture conditions optimized to quadruplicate FAE production. Purification and sequencing of the FAE protein allowed to approach PCR amplification of its genomic DNA and to sequence the FAE encoding gene. RT-PCR of total RNA revealed no other co-transcribed genes downstream or upstream of the FAE gene. Furthermore, different culture conditions gave an insight into transcriptional repression of the gene by glucose.

Conclusions

S. werraensis produces a FAE, which achieves maximal yields within shortest incubation times using inexpensive carbon and nitrogen sources, showing activity on natural substrates but no dimerization of FA. *S. werraensis* is an interesting candidate for upscaling fermentation aimed on benefiting from both, the broad range of applications of the enzyme itself and the production of FA for further processing.

FEMS7-1608
Physiology / Biochemistry / Molecular Microbiology - Part III

FIGHTING BURKHOLDERIA CENOCEPACIA THROUGH A NEW PROMISING BACTERICIDAL MOLECULE

V.C. Scoffone[1], A.S. Gislason[2], A.M. Hogan[2], L.R. Chiarelli[1], M.S. Stietz[2], A. Azzalin[3], V. Makarov[4], S.T. Cardona[2], G. Riccardi[1], S. Buroni[1]
[1]University of Pavia, Biology and Biotechnology, Pavia, Italy
[2]University of Manitoba, Department of Microbiology, Winnipeg, Canada
[3]Università di Pavia, Neurosurgery- Dipartimento di Scienze Clinico-Chirurgiche e Pediatriche- Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy
[4]Research Center of Biotechnology of the Russian Academy of Sciences, Bach Institute of Biochemistry, Moscow, Russia

Backgrounds

The respiratory tract of Cystic Fibrosis patient can be colonized by *Burkholderia cenocepacia*, a Gram-negative opportunistic bacterium characterized by a high level of antibiotic resistance. Exploration of molecular mechanisms essential for pathogen survival are essential to identify new molecular targets and new drugs for the treatment of these infections.

Objectives

We previously identified a new molecule belonging to the 2,1,3-benzothiadiazol-5-yl family (C109) effective against *Burkholderia cenocepacia* (MIC = 8 µg/ml). The compound is active against clinical isolates and other members of the Bcc, as well as against other Gram-negative and -positive bacteria. Our aims are the characterization of this molecule, the identification of its target and the elucidation of the related molecular mechanism.

Methods

By a chemogenomic approach, time-lapse fluorescence microscopy and biochemical assays the mechanism of action of C109 was shown to rely on the inhibition of the divisome machinery. Toxicity experiments carried out on CF epithelial bronchial cells revealed low toxicity of the compound.

Conclusions

In summary, we characterized the molecular mechanism of a new compound active against *B. cenocepacia*, identifying the cellular target as the bacterial divisome.
The final aim of our work is to characterize the *B. cenocepacia* divisome machineries and to develop C109 derivatives more effective. This could improve and extend CF patient lives fighting infections due to *B. cenocepacia*, paving the way for the development of new therapies.

FEMS7-1835
Physiology / Biochemistry / Molecular Microbiology - Part III

AMINO ACID CATABOLISM OF THERMOANAEROBACTER AND CALDANAEROBACTER SPECIES

S. Scully[1], *J. Orlygsson*[1]
[1]*University of Akureyri, Natural Resource Science, Akureyri, Iceland*

Backgrounds

Thermoanaerobacter strains have been widely investigated for biofuel production from carbohydrates while proteins and amino acids catabolism have received less attention. Amino acid catabolism is challenging under anaerobic conditions unless hydrogen is removed. Recent reports have demonstrated that *Thermoanaerobacter* strains can degrade branched-chain amino acids (BCAAs) to a mixture of their corresponding fatty acids (BCFAs) and alcohols (BCOHs) when thiosulfate is present.

Objectives

The aim of the presented work to evaluate the ability of these genera to degrade amino acids and better understand the mechanisms dictating the observed end product ratios.

Methods

The type species of the genera of *Thermoanaerobacter* and *Caldanaerobacter*, were investigated in batch culture for each of the proteogenic amino acids with and without the addition of thiosulfate as an electron acceptor.

Conclusions

Some strains in the genus could utilize serine and threonine without the addition of thiosulfate with acetate and ethanol being the dominate products while BCFA/BCOH ratios from BCAA were highly dependent on species and substrate when thiosulfate was added. BCAA catabolism resulted mixtures of the corresponding BCOH (0.5-5 mM) and BCFA (3.5-15.5 mM) with partial substrate utilization being observed in some cases. Altering the liquid-gas phase ratio had an impact on end product ratios; in the case of *C. subterraneus* subsp*. yonseiensis,* increasing the L-G ratio from 1.00 to 5.88, the BCOH formation increased more than 5-fold (with thiosulfate) and less BCFA were produced. Preliminary results on pathway elucidation studies using enzymatic assays and ^{13}C NMR studies will also be presented.

FEMS7-0948
Physiology / Biochemistry / Molecular Microbiology - Part III

EFFECT OF QUINONE CONCENTRATION ON MEMBRANE FLUIDITY IN LISTERIA MONOCYTOGENES ISOLATES FROM FOOD AT LOW TEMPERATURES

W. Seel[1], A. Lipski[1]
[1]Institut für Ernährungs- und Lebensmittelwissenschaften, Lebensmittelmikrobiologie und -hygiene, Bonn, Germany

Backgrounds

Listeria monocytogenes is well known as a food pathogen capable of growing at a broad temperature range, from 50 °C down to refrigerator temperatures. A key function in bacterial survival at low temperatures is their ability to adjust lipid composition in order to maintain membrane fluidity. In *L. monocytogenes* this is accomplished by modification of fatty acid chain length. Preliminary analyses showed different extent of fatty acid profile adaption for *L. monocytogenes* strains, which indicates the presence of a second adaptation mechanism.

Objectives

For eight *L. monocytogenes* isolates from food and two reference strains from culture collections (*L. monocytogenes* DSM 20600^T and ATCC 19115) the fatty acid profiles were analyzed as function of growth temperature (37°C versus 6°C). In addition the quinone concentrations were determined.

Methods

Membrane fluidity was analyzed *in vivo* by measuring generalized polarization and anisotropy with the fluorescent dyes laurdan and TMA-DPH, respectively.

Conclusions

In this study, 7 out of 10 strains showed a strong increase of the ai-15:0/ai-17:0 ratio up to 20-fold at low temperature and a slight decrease in quinone concentration. Three strains showed, contrary to expectations, only a small shift in ai-15:0/ai-17:0 ratio but an increased quinone content at 6 °C. These strains with quinone-enriched membranes exhibited a significantly different membrane fluidity characteristic with a more expanded phase transition zone in contrast to the strains with decreased quinone content. The broadening of the phase transition zone by increasing of non-fatty acid membrane lipids is discussed as an additional adaptation mechanism to temperature shifts for *Listeria monocytogenes* strains.

FEMS7-0887
Physiology / Biochemistry / Molecular Microbiology - Part III

REACTIVE OXYGEN SPECIES-INDEPENDENT ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES THROUGH INNER MEMBRANE DYSFUNCTION ON THE GRAM-NEGATIVE BACTERIUM SALMONELLA ENTERICA SP. TYPHIMURIUM

M. Seong[1], *D.G. Lee*[1]
[1]*Kyungpook National University, School of life sciences, Daegu, Republic of Korea*

Backgrounds

Silver nanoparticles (AgNPs) are one of silver substance that has been used as an antibacterial agent in various fields. Although the antibacterial activity of AgNPs against gram negative bacteria have been studied previously, the mechanism of AgNPs against *S. typhimurium* remains unclarified.

Objectives

We revealed the mode of action of AgNPs against gram-negative bacterium *S. typhimurium* through activity of cell death related experiments at various concentrations.

Methods

To evaluate the inhibitory effects on AgNPs induced bacterial cell death, reactive oxygen species (ROS) accumulation were monitored various range of concentrations using H_2DCFDA. Cell viability and the cellular response to calcium ion signaling was measured after pretreatment with N-acetylcysteine (NAC). N-phenyl-1-naphthylamine assay and *O*-nitrophenyl-β-D-galactopyranoside assay measured outer membrane and inner membrane permeability respectively by spectrofluorophotometer.

Conclusions

AgNPs induces ROS-independent antibacterial activity which is most affected by inner membrane dysfunction on gram negative bacterium *S. typhimurium.*

FEMS7-0579
Physiology / Biochemistry / Molecular Microbiology - Part III

SALT STRESS AFFECTS COMPLEMENTATION OF THE SOLUBLE CYTOSOLIC PYROPOSPHATASE OF YEAST BY FUNCTIONALLY DIFFERENT MEMBRANE-BOUND ION-TRANSLOCATING PYROPHOSPHATASES

J.R. Pérez-Castiñeira[1], J.M. Madroñal[1], A. Hernández[2], A. Serrano[1]
[1]Inst. de Bioquímica Vegetal y Fotosíntesis- CSIC-Univ. de Sevilla, Bioenergética del Fosfato, Sevilla, Spain
[2]Instituto de Ciências Biomédicas- Universidade de São Paulo, Depto. Parasitologia, 05508-000 São Paulo SP, Brazil

Backgrounds

Overexpression of the membrane-bound K^+-dependent H^+-translocating inorganic pyrophosphatase (H^+-PPase) from higher plants has been reported to alleviate the sensitivity towards NaCl in these organisms (PNAS USA 2001, 98:11444). This strategy had been initially tested in *Saccharomyces cerevisiae* (PNAS USA 1999, 96:1480), thus confirming this microorganism as a good model to study and test solutions to this and other stresses in plants.

Objectives

We have previously shown that membrane-bound PPases can functionally complement the cytosolic soluble pyrophosphatase (Ipp1p) of the yeast *Saccharomyces cerevisiae* (Biochem J 2010, 42:147; PNAS USA 2002, 99:15914). Here, the capacity of the former proteins to complement Ipp1p under salt-stress has been studied using several yeast strains with different sensitivities towards NaCl.

Methods

Three membrane-bound PPases were tested: the vacuolar K^+-dependent H^+-PPase AVP1 from the higher plant *Arabidopsis thaliana* (AVP1), the K^+-dependent Na^+-PPase from the halotolerant methanogenic archaea *Methanosarzina mazei* (MVP) and the K^+-independent H^+-PPase from the photosynthetic proteobacterium *Rhodospirillum rubrum*. The archaeal Na^+-PPase consistently supported yeast growth more efficiently than both plant and bacterial H^+-PPases under salt stress. *In vitro* studies showed that both hydrolytic and H^+-pumping activities of AVP1 are remarkably sensitive to Na^+. Moreover, comparative studies of sensitivity of PPase activity to Na^+ showed that the archaeal Na^+-PPase was expectedly less sensitive to this cation than its H^+-pumping counterparts.

Conclusions

We propose that membrane-bound PPases may be direct targets of Na^+, consequently, the strategy of using these proteins in order to improve salt-tolerance in plants might be significantly improved if Na^+-PPases are utilised for this purpose.

FEMS7-0581
Physiology / Biochemistry / Molecular Microbiology - Part III

WIDE OCCURRENCE AND PHYSIOLOGICAL RELEVANCE OF SODIUM-PUMPING PYROPHOSPHATASES IN MARINE PROTISTS

J.M. Madroñal[1], J.R. Pérez-Castiñeira[1], A. Serrano[1]
[1]Inst. de Bioquímica Vegetal y Fotosíntesis- CSIC-Univ. de Sevilla, Bioenergética del Fosfato, Sevilla, Spain

Backgrounds

Ion (H^+/Na^+)-translocating pyrophosphatases (m-PPases) are integral proteins of prokaryotic cellular and eukaryotic vacuo-lysosomal membranes coupling hydrolysis of energy-rich pyrophosphate, a ubiquitous subproduct of anabolism, to the generation of electrochemical gradients. Many protistan genomes have set of genes encoding functionally diverse m-PPase paralogs.

Objectives

We aim to identify and functionally characterize the putative Na^+-translocating m-PPases (Na^+-PPases) encoded by the genomes of phylogenetically diverse marine microalgae (prasinophytes, stramenopiles, haptophytes, chlorarachniophytes, rhodophytes, dinophytes).

Methods

Our studies with the diatom *Phaeodactylum tricornutum*, the prasinophyte *Ostreococcus tauri* and the rhodophyte *Porphyridium purpureum* indicate that do contain functional Na^+-PPases which are less sensitive to ionic stress than their H^+-pumping counterparts. An active role of Na^+-PPases in ion homeostasis of marine protists is consistent with the abundance of their transcripts in EST libraries and the Marine Microbial Eukaryotic Transcriptome Sequencing Project (MMETS). The subcellular location of microalgal Na^+-PPases is of paramount importance to discern if they are either the first primary sodium pumps found so far in endomembranes or the only sodium pumps of the eukaryotic cell membrane able to use a substrate alternative to ATP.

Conclusions

These m-PPases are the first Na^+-PPases reported in eukaryotes, and they probably arose via HGT given its remarkable amino acid sequence similarity to their prokaryotic orthologs of bacteria and archaea. Moreover, their wide distribution among marine protists suggests an important physiological role for these molecular pumps in saline habitats. Eukaryotic Na^+-PPases should play a pivotal role in cations homeostasis of marine protists, which are poikilosmotic organisms that use sodium-dependent co-transporters for nutrients uptake.

FEMS7-1679
Physiology / Biochemistry / Molecular Microbiology - Part III

FURC OVEREXPRESSION LEADS TO VARIATIONS IN THE COMPOSITION AND EFFICIENCY OF PHOTOSYNTHETIC APPARATUS IN ANABAENA PCC7120

E. Sevilla[1], C. Sarasa[1], E. Broset[1], R. Cases[2], M.L. Peleato[1], M.F. Fillat[1]
[1]Faculty of Sciences and Biocomputation and Complex Systems Physics Institute BIFI- University of Z aragoza,
Department of Biochemistry and Molecular and Cell Biology- Biochemical- Biophysical and Computati onal Biology Group - CSIC Joint Unit, Zaragoza, Spain
[2]ICMA, Functional Organic Materials, Zaragoza, Spain

Backgrounds

Ferric uptake regulator (Fur) proteins are transcriptional regulators that play key roles in many prokariots. *Anabaena* PCC 7120 contains in its genome three paralogs of Fur proteins whose genes are called *all1691* (*furA*), *all2473* (*furB*) and *alr0954* (*furC*). Until now, little is known about the role of FurC in cyanobacteria.

Objectives

This work was focused on the study of the functions of FurC protein in *Anabaena* PCC7120. In order to achieve this goal, an overexpression mutant of FurC protein was constructed in *Anabaena* PCC7120 rendering the EB2770 strain.

Methods

Scanning electron microscopy (SEM) studies and growth curves were performed comparing the EB2770 and the wild type strains. Photosynthetic pigments were also determined. Whole-cell absorbance spectra and the fluorescence emission spectra at 77K were carried out in order to compare the composition and efficiency of photosynthetic apparatus in both strains. Finally, pull down experiments were performed with purified FurC protein to identify proteins that could interact with this protein.

Conclusions

SEM studies revealed changes in size, shape and roughness of the EB2770 cells. Pigment content and yield of energy transfer between the phycobilisome and the photosystem I and II was notably altered in the EB2770 mutant. Pull down results indicated that two linker proteins of phycobilisome interacted with FurC protein. These results suggest that FurC has a role regulating the final composition of photosynthetic pigments and therefore influencing the yield of energy transfer between the phycobilisome and the photosystem I and II although the detailed mechanism remains unknown.

FEMS7-0086
Physiology / Biochemistry / Molecular Microbiology - Part III

AMINO ACID SUBSTITUTION OF ACRB CONTRIBUTES TO AZITHROMYCIN RESISTANCE IN HAEMOPHILUS INFLUENZAE

S. Seyama[1], T. Wajima[1], H. Nakaminami[1], N. Noguchi[1]
[1]Tokyo University of Pharmacy and Life Sciences, Microbiology, Tokyo, Japan

Backgrounds

Macrolides, including azithromycin (AZM) and clarithromycin (CAM), can be used as alternatives of β-lactams for the treatment of *Haemophilus influenzae* infections. However, CAM-resistant *H. influenzae* strains have been isolated frequently in Japan. We showed that a nonsense mutation in the negative regulator gene *acrR* of these isolates resulted in overexpression of chromosomal efflux pump AcrAB, which is the underlying mechanism of the resistance. A nonsense mutation of *acrR* conferred resistance to CAM but not AZM.

Objectives

In this study, we isolated AZM-resistant strains and analysed the mechanisms underlying AZM resistance.

Methods

A total of 155 *H. influenzae* isolated during 2010–2012 were used in this study. The *acr* operon of these isolates was analysed by DNA sequencing. The transcription levels of chromosomal multidrug efflux pumps were evaluated by semiquantitative reverse-transcription PCR. A transformation experiment was performed by electroporation using *H. influenzae* Rd. Antimicrobial susceptibilities of the isolates were tested using the broth-dilution method.

Conclusions

A strain (2012-42) resistant to both AZM and CAM was found by antimicrobial susceptibility testing. An elevated transcript level of *acrB* resulting from the nonsense mutation in *acrR* was observed in the strain 2012-42, as well as in the strain 2011-70 that was resistant to CAM but not AZM. We found by sequencing of the *acr* region that the strain 2012-42 had a specific amino acid substitution Arg327Ser in AcrB. To confirm that the amino acid substitution in AcrB contributes to AZM resistance, the *acr* region of the strain 2012-42 was introduced into *H. influenzae* Rd. The MICs of AZM for the transformants ($Rd_{2012\text{-}42}$) increased to the same level as that of the donor strain.
Our findings strongly suggested that the 327th amino acid substitution of AcrB contributes to AZM resistance, in addition to nonsense mutation in *acrR*.

FEMS7-0020
Physiology / Biochemistry / Molecular Microbiology - Part III

ANTIMICROBIAL RESISTANCE AND MOLECULAR PREVALENCE OF EFFLUX PUMP ENCODING GENES (ADER, ADEB) IN ACINETOBACTER BAUMANNII STRAINS ISOLATED FROM HOSPITALIZED PATIENTS IN TEHRAN

S. Shabani[1], P. Saffarian[2], R. Mirnejad[3]
[1]Tehran University of Medical Sciences school of Nursing and Midwifery, science, Tehran, Iran
[2]Tehran Medical Branch- Islamic Azad University- Tehran- Iran., Microbiology, Tehran, Iran
[3]Baqiyatallah University of Medical Sciences, Molecular Biology Research Center, Tehran, Iran

Backgrounds

Acinetobacter baumannii causes variety of nosocomial infections which often lead to death. Because of wide range drug resistance amongst A. baumannii strains, it becomes a problem in clinical treatments. One of the main factors causing resistance in A. baumannii strains is the efflux pump.

Objectives

The aim of this study was to determine the prevalence of adeR and adeB genes encoding efflux pump between A. baumannii strains isolated from hospitalized patients in Tehran.

Methods

300 clinical samples were collected from some main hospitals in Tehran. After strain determination through biochemical and culture method, antimicrobial resistance of A. baumannii isolates was evaluated with dick diffusion agar. Finally, the presence of adeR and adeB genes were examined using molecular method, PCR.

Conclusions

Out of 300 clinical samples, 100 A. baumannii isolates were determined. 70% of A. baumannii isolates showed resistance against three or more antibiotics. Moreover, molecular method assessment revealed that the prevalence of either adeR or adeB genes among antibiotic resistance A. baumannii strains is 96%.
According to results of this study, the efflux pump encoding genes (adeR and adeB) are active in between antibiotic resistance A. baumannii strains.

FEMS7-0151
Physiology / Biochemistry / Molecular Microbiology - Part III

ATP DEPENDENT PERSISTER FORMATION IN ESCHERICHIA COLI

Y. Shan[1], *K. Lewis*[1]
[1]*Northeastern University, Antimicrobial Discovery Center, Boston, USA*

Backgrounds

Persisters are a subpopulation of cells that are tolerant to antibiotics. Persisters have been shown to be responsible for recalcitrance of chronic infections. In *Escherichia coli,* one widely-accepted model of persister formation holds that stochastic accumulation of ppGpp causes activation of the Lon protease that degrades antitoxins; active toxins then inhibit translation, resulting in dormant, drug-tolerant persisters.

Objectives

In this study we systematically examined induction of mRNA interferase encoding toxin-antitoxin (TA) systems upon stress. We found that various stresses induce mRNA interferase encoding TA expression, however, induction of these TAs does not necessarily increase persisters. The 16S ribosomal RNA promoter *rrnB* P1 was proposed to be a persister reporter and an indicator of toxin activation. We investigated the role of toxin activation in persister formation.

Methods

Using FACS, we confirmed the enrichment for persisters in *rrnB* P1-*gfp* dim cells after antibiotic treatment, however, this is independent of toxin-antitoxins. *rrnB* P1 is co-regulated by ppGpp and ATP. We show that *rrnB* P1 can report persisters independent of ppGpp, suggesting that *rrnB* P1 is a persister marker responding to ATP.

Conclusions

Cells surviving antibiotic treatment exhibit lower intracellular ATP levels. Consistent with these findings, decreasing the level of ATP by arsenate causes drug tolerance. Lowering ATP slows translation and prevents formation of DNA double strand breaks upon fluoroquinolone treatment, indicating that decreased antibiotic target activity is the reason for persister formation. We conclude that variation of ATP levels leads to persister formation through decreasing antibiotic target activity.

FEMS7-0152
Physiology / Biochemistry / Molecular Microbiology - Part III

INVESTIGATION OF ESCHERICHIA COLI PERSISTERS TOLERANT TO B-LACTAMS USING TN-SEQ

Y. Shan[1], *K. Lewis*[1]
[1]*Northeastern University, Antimicrobial Discovery Center, Boston, USA*

Backgrounds

Persister are dormant variants that form a subpopulation of drug tolerant cells largely responsible for recalcitrance of chronic infections. In *E. coli*, level of persisters tolerant to a β-lactam antibiotic increase dramatically upon reaching stationary phase due to a cessation of cell growth, however, the molecular mechanism of this phenotype is unknown.

Objectives

In this study, we applied transposon sequencing (Tn-Seq) to identify mutants with a low level of persisters tolerant to ampicillin in stationary phase.

Methods

We utilized a highly-saturated transposon library and exposed a stationary phase culture to a high dose of ampicillin.

Conclusions

We identified 28 mutants with significantly decreased survival after ampicillin treatment. One of the high hits is *murR*, a negative regulator for peptidoglycan recycling. Deletion of *murR* may decrease stationary phase survival by promoting cell wall remodeling in stationary phase, and thus serves as a positive control for the screen. We also identified five genes encoding enzymes involved in central metabolism and one gene encoding a periplasmic peptidase. These findings demonstrate that central metabolism reprogramming plays an important role in the complete tolerance of stationary phase *E. coli* to β-lactams. On-going work aims at elucidating the metabolic switch involved in the process and will lead to a comprehensive understanding of β-lactam tolerance.

FEMS7-1496
Physiology / Biochemistry / Molecular Microbiology - Part III

UNSATURATATION OF MEMBRANE LIPIDS AND THEIR COMPOSITION OF YEAST WICKERHAMOMYCES ANOMALUS B59 A POSSIBLE KEY FACTOR IN RESPONSE TO FURFURAL STRESS

S.C. Sharma[1]
[1]*Panjab University, Biochemistry, Chandigarh, India*

Backgrounds

Most important biofuel at world level is ethanol. The latter derived from agricultural lignocellulosic residues is a promising alternative to fossil fuel for energy based needs Plasma membrane is the primary target of various stresses. Furfural is present in post harvest lignocellulosic materials after acid hydrolysis of xylan and has been shown to exert an inhibitory effect in the process of fermentation.

Objectives

To analyse lipid changes in response to furfural stress; a key bye product of lignocellulosic acid hydrolysates in yeast cells.

Methods

Isolated strain of Wickerhamomyces was grown in YPD medium with/without furfural 10mM. Lipids extracted by Bligh& Dyer; phospholipids;sterols; glycolipids; by Ames(1966) ; Sperry& Webb: Dubois(1950) procedures fatty acids by GCMS respectively. Individual phospholipids were separated by 2DTLC. Fluidity was determined by DPH and TMA-DPH probes

Conclusions

The amount of phospholipids decreased while that of glycolipids and total sterols increased. The increase in free sterol: phospholipid (molar ratio) and glycolipids : phospholipids ratio showed decrease in membrane fluidity. Among the individual phospholipids: phosphatidylethanolamine and phosphatidylcholine levels increased while that of phosphatidylserine showed an opposite trend. No change was observed in the amount of phosphatidylinositol. The ratio of unsaturated to saturated fatty acids decreased and that of short chain: long chain showed about 40% increase. The results showed importance and implications of unsaturated lipids molecules and thereby membrane fluidity changes not only in the presence of ethanol but to exposure to furfural also.

FEMS7-1029
Physiology / Biochemistry / Molecular Microbiology - Part III

GENOME AND PROTEOME OF THE HUMAN INTESTINAL CORE BACTERIUM EUBACTERIUM HALLII

S. Shetty[1], T.P. Nam Bui[1], S. Aalvink[1], S. Boeren[2], J. Ritari[3], L. Paulin[4], W. M. de Vos[1,3,5]
[1]Wageningen University and Research, Laboratory of Microbiology, Wageningen, The Netherlands
[2]Wageningen University and Research, Laboratory of Biochemistry, Wageningen, The Netherlands
[3]University of Helsinki,
Department of Veterinary Biosciences- Division of Microbiology and Epidemiology, Helsinki, Finland
[4]Institute of Biotechnology- University of Helsinki, DNA Sequencing and Genomics Laboratory, Helsinki, Finland
[5]University of Helsinki,
RPU Immunobiology- Department of Bacteriology and Immunology- Faculty of Medicine, Helsinki, Finland

Backgrounds

Eubacterium hallii is an anaerobic butyrate-producing bacterium commonly found in the human gut and part of its core microbiome (*Shetty et al. FEMS Microbiol Rev 2017 in press*). Oral administration of *E. hallii* strain L2-7 has been found to improve insulin sensitivity in mice models and hence this is a candidate for next generation microbiome therapeutics (*Udayappan et al. NPJ Biofilms & Microbiome 2016, 16009*).

Objectives

To improve our understanding of the physiology and functional capabilities of *E. hallii* strain L2-7.

Methods

The genome was sequenced using PacBio RII and HiSeq2000 instruments, and its proteome was obtained by nLC–MS/MS with a Proxeon EASY nLC and a LTQ-Orbitrap XL mass spectrometer.

Conclusions

The 3.5 Mb-genome of *E. hallii* strain L2-7 is predicted to encode 3226 proteins and 89 RNAs. The latter includes eight 16S rRNA genes with a high level of sequence diversity that are widely present in the human HMP gut database. Proteome analysis showed genes involved in butyrate production *via* the acetyl-CoA pathway to be highly expressed, with the second most dominant protein being butyryl-CoA dehydrogenase. In addition, the canonical proteins involved in a bacterial microcompartment were found to be present in high amounts, which is compatible with the experimental detection of vitamin B12 and several pathways with reactive metabolites where this cofactor plays a role. Comparative analysis of genomes and proteomes of closely related *Eubacterium* species will be presented as to identify unique features of the strain L2-7 and its relevance for human health.

FEMS7-1023
Physiology / Biochemistry / Molecular Microbiology - Part III

CYANOBACTERIAL BIOACTIVE COMPOUNDS FROM BRAZILIAN UNDER-EXPLORED ENVIRONMENTS

T. Shishido Joutsen[1], J. Jokela[2], M. Wahlsten[3], D.P. Fewer[3], M.F. Fiore[4], L. Herfindal[5], P. Permi[6], K. Sivonen[3]
[1], Espoo, Finland
[2]University of Helsinki, Food and Environmental Sciences, Helsinki, Finland
[3]University of Helsinki, Department of Food and Environmental Sciences, Helsinki, Finland
[4]University of São Paulo, Center for Nuclear Energy in Agriculture, Piracicaba, Brazil
[5]University of Bergen, Department of Clinical Science and Centre for Pharmacy, Bergen, Norway
[6]University of Jyväskylä,
Department of Chemistry- Department of Biological and Environmental Science, Jyväskylä, Finland

Backgrounds

Brazil has a vast territory in South America which comprises different types of biomes, including Amazon, Caatinga, Pantanal and Cerrado. These diverse environments are characterized by high biodiversity. Cyanobacteria are photosynthetic prokaryotes found in diverse habitats.

Objectives

In this study, we analyzed cyanobacterial isolates from Brazilian under-explored environments to improve the knowledge of antimicrobial and anticancer compounds produced by these strains.

Methods

A total of 62 cyanobacterial isolates were tested against opportunistic fungi and bacteria (e.g. *Candida albicans*, *Staphylococcus aureus* and *Salmonella typhimurium*) and leukemia (MOLM-13) and normal (NRK) cell lines. Most of the cell extracts had anticancer activity (63%), followed by antibacterial (26%) and antifungal (11%) activities. Known bioactive compounds were detected using LC-MS and Q-TOF in 12 of the studied strains, including microcystins, hassallidins and puwainaphycins. Unknown bioactive compounds responsible for the observed activities are currently being isolated from crude methanol extract by HPLC. Preliminary results indicate that CENA513 and CENA514 produce a bacteriostatic compound and a bioactive compound with potent anticancer activity. CENA71, CENA72 and CENA302 produce hydrophobic antibacterial compound(s). CENA69 showed to produce a very potent cytotoxic compound. Further analysis will be performed to isolate and chemically characterize the bioactive compounds. We have sequenced the genomes of two strains and the genome mining is aiding in the discovery of the yet to known bioactive compounds.

Conclusions

This study will improve the knowledge about the genetic pathway involved in the compounds synthesis and the metabolites produced by cyanobacteria isolated from Brazilian environments.

FEMS7-1949
Physiology / Biochemistry / Molecular Microbiology - Part III

THE PUTATIVE TYPE I SECRETION SYSTEM HGDB/HGDC IS ESSENTIAL FOR PROPER FUNCTIONALITY OF ANABAENA PCC7120 HETEROCYSTS

D. Shvarev[1], *C. Nishi*[1], *L. Wörmer*[2], *I. Maldener*[1]
[1]*Eberhard Karls University of Tübingen, IMIT Organismic Interactions, Tübingen, Germany*
[2]*University of Bremen,*
Organismic Geochemistry- MARUM – Center for Marine Environmental Sciences, Bremen, Germany

Backgrounds

The filamentous cyanobacterium *Anabaena* PCC7120 develops specific cells, called heterocysts, which arise in filaments during nitrogen starvation. The heterocysts fix atmospheric nitrogen and supply vegetative cells with nitrogen compounds. They create a micro-oxic environment to protect the nitrogenase complex from external oxygen by deposing a special cell envelope, which consists of two layers: the thick polysaccharide layer and the heterocyst specific glycolipid (HGL) layer. Previously we showed that *Anabaena* PCC7120 uses a specific type I secretion system (T1SS), for the export of HGLs into the cell envelope (Staron et al., 2011). This transporter comprises four different oligomeric proteins: the outer-membrane protein TolC/HgdD, the membrane fusion protein DevB, the inner membrane factor DevC and the ATPase DevA.

Objectives

To investigate a possible role of other T1SS in heterocyst differentiation we performed a mutational analysis in genes, which are homologous to genes of several subunits of the DevBCA complex. In this work we focused on functions of HgdB (*all5347*) and HgdC (*all5346*), which are homologues to DevB and DevC*,* respectively (see also Fan et al., 2005).

Methods

We mutated the *hgdB*- gene and re-investigated the phenotype using light and fluorescent microscopy, TEM and various analytical chromatographical methods.

Conclusions

The mutant in this T1SS is not able to form mature heterocysts and to grow on N_2 as a sole nitrogen source. We will present and discuss data on aberrant envelope formation, nitrogen fixation and the localization of the HgdC protein by GFP fusion and will show that HgdB/D is important for heterocyst HGL formation in *Anabaena*.

FEMS7-2679
Physiology / Biochemistry / Molecular Microbiology - Part III

HOW DO BACTERIA MAKE PROTEINS? UNDERSTANDING THE BIOGENESIS OF C-TYPE CYTOCHROMES

A. Silva[1], A. Veríssimo[2], F. Daldal[3], R.O. Louro[1]
[1]Instituto de Tecnologia Química e Biológica António Xavier, Biochemestry, Oeiras, Portugal
[2]University of Texas, Biochemestry, El Paso, USA
[3]University of Pennsylvania, Biology, Philadelphia, USA

Backgrounds

Cytochromes-*c* are essential proteins for living organisms across all domains of life. These proteins are ubiquitous and play key roles in important biological processes, from electron transfer to apoptosis. These proteins are also involved in several bioenergetic processes, having crucial importance in bioremediation and biotechnological applications. *C*-type cytochromes are metalloproteins that contain heme(s) covalently bound to the polypeptide chain via 2 thioether bonds to cysteine side chains. Given the importance of this class of proteins, the process that leads to their formation in nature is equally important. The covalent attachment of heme and subsequent folding of the protein requires a dedicated maturation machinery. Despite the recognized biological importance and biotecnological applications of *c*-type cytochromes, this process is far from fully understood.

Several maturation systems have been described, including the Cytochrome-*c* maturation (Ccm) System I. This system is present in most Gram-negative bacteria, including the genus *Escherichia*, *Vibrio* and *Shewanella*. These are specially important as model organisms, pathogens and electroactive bacteria.

Objectives

The aim of this work is to characterize the cytochrome-*c* maturation system I (Ccm system).

Methods

Using isotopic labelling, NMR approaches and site directed mutagenesis, we aim to characterize protein interactions and recognition mechanisms between the substrates and components of System I to understand the functioning of this system.

Conclusions

This work will characterize how the apocytochrome *c* chaperone CcmI, recognizes different classes of substrates (apocytochromes *c*). This will provide unique insights on the nature of these interactions and structure of the interacting domains providing a full understanding on cytochrome biogenesis in bacteria.

FEMS7-3176
Physiology / Biochemistry / Molecular Microbiology - Part III

MOLECULAR EPIDEMIOLOGY OF MDR ACINETOBACTER BAUMANNII FROM THREE TERTIARY CARE HOSPITALS FROM DIFFERENT REGIONS OF INDIA

L. Singhal[1], S. Kumar[2], U. Devi[3], V. Gautam[2], J. Chander[1], P. Ray[2]
[1]Government Medical College & Hospital, Microbiology, Chandigarh, India
[2]Post Graduate Institute of Medical Education & Research, Medical Microbiology, Chandigarh, India
[3]Regional Medical Research Centre, Microbiology, Dibrugarh, India

Backgrounds

Acinetobacter baumannii is a multidrug-resistant (MDR) nosocomial pathogen associated with healthcare-associated infections (HAIs). The organism's ability to survive in different environment for a prolonged period of time and its resistance to desiccation & disinfectants make it difficult to eradicate. Circulation among hospitalized patients through hospital settings is of particular concern in development of MDR *A. baumannii*.

Objectives

We studied the molecular epidemiology of clinical isolates of MDR *A. baumannii* from three major hospitals of India.

Methods

A total of 20 isolates of *A. baumannii* will be included in this study. 8 isolates each from two hospitals of North India, and 4 isolates from a North-East India will be subjected to MLST typing. *A. baumannii* species were identified with MALDI-TOF. Antimicrobial susceptibility for a set of antimicrobials was tested using disc diffusion method. MLST^{OX} was performed for isolates from North India and MLST of North-East India is being processed. From one Hospital (GMCH), out of 8 isolates, 3 isolates represented a dominating ST-231 belonging to International Clone-I (IC-I) and 4 were assigned new STs (1387, 1388, 1389 & 1390). Another hospital (PGIMER), showed 3 new STs (859, 1050 & 1051) and one isolate (ST 447) found belonging to IC-II.

Conclusions

In India, this is the first study of its kind, where we have reported 2 International Clones circulating in two North Indian hospitals. MLST results of third centre will have the better epidemiology of this bug. Another large-scale study would promisingly give a better picture of hospital-wide spread of different clones of *A. baumannii*.

FEMS7-2602
Physiology / Biochemistry / Molecular Microbiology - Part III

IDENTIFICATION OF A NOVEL GENE INVOLVED IN BETA-LACTAM RESISTANCE IN STAPHYLOCOCCUS AUREUS

W. Sirisarn[1], *G. Xia*[1]
[1]*Division of Infection- Immunity and Respiratory Medicine,*
Faculty of Biology- Medicine and Health- The University of Manchester, Manchester, United Kingdom

Backgrounds

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a serious issue in clinical environments around the world. Beta-lactam resistance in MRSA depends on the transpeptidase activity of low-affinity penicillin binding protein, PBP2A, which is encoded by the *mecA* gene. In addition, resistance also requires certain 'auxiliary factors' such as *fmtA, tagO* and others.

Objectives

we aim to identify and characterise novel auxiliary factor genes, which help understand beta-lactam resistance mechanisms, and may provide new strategies to combat MRSA infections.

Methods

Using transposon mutagenesis, we identified a novel auxiliary factor, *auxB*. *auxB* mutants with different MRSA genetic backgrounds were constructed by phage transduction and allelic exchange. The beta-lactam susceptibility of these mutants were analysed by measuring the MIC of various beta-lactam antibiotics.

Conclusions

Transposon mutation or deletion of *auxB* causes a 2-32 fold reduction in the minimum inhibitory concentration (MIC) of cefoxitin, oxacililin, ceftazidime, cephradine and meropenem, and complementation restores these MICs back to the wild type level, suggesting auxB is required for beta-lactam resistance in MRSA.

FEMS7-2082
Physiology / Biochemistry / Molecular Microbiology - Part III

MARINOBACTER HYDROCARBONOCLASTICUS SP17 BIOFILM FORMATION ON HYDROPHOBIC ORGANIC CARBON INVOLVES SIGMA 54-MEDIATED TRANSCRIPTIONAL REGULATION OF PUTATIVE EXPORTED PROTEINS

P. Sivadon[1], *I. Guefrachi*[1], *F. Hakil*[1], *L. Urios*[1], *R. Grimaud*[1]
[1]*IPREM UMR CNRS 5254, Microbial Ecology, Pau Cedex, France*

Backgrounds

Marinobacter hydrocarbonoclasticus SP17 (*Mh*SP17) degrades a wide range of hydrophobic organic carbon (HOC) through an oleolytic biofilm formed at hydrocarbon/lipid-water interfaces. The matrix of this oleolytic biofilm is mainly composed of proteins, some of which are being involved in the assimilation of hydrophobic substrates.

Objectives

To identify and characterize exported proteins that play in early steps of oleolytic biofilm formation and hydrocarbon/lipid assimilation.

Methods

Whole genome transcription analyses were conducted on early stages of biofilm formation on metabolizable HOCs (hexadecane or triolein) and on heptamethylnonane, a non metabolizable isomer of hexadecane. Although being able to adhere to the heptamethylnonane/water interface, *Mh*SP17 does not develop micro-colonies and subsequent biofilm, even in the presence of acetate as supplemental carbon source. Functional genomics was coupled to physiological studies in order to test the involvement of differentially expressed genes coding putative exported proteins on oleolytic biofilm formation.

Conclusions

Several genes expressing proteins with a common putative export signal showed similar overexpression patterns on metabolizable HOCs. Mutagenesis of the corresponding protein export system resulted in reduced oleolytic biofilm growth. These genes also carry a sigma 54-RpoN binding sequence in their promoter sequences and we could show that RpoN regulates their expression. Consistently, *rpoN* mutant exhibited severe reduction in cell adhesion to HOC/water interface and in oleolytic biofilm growth. Consequently, the capacity of *Marinobacter hydrocarbonoclasticus* SP17 to form oleolytic biofilms on hydrocarbons and lipids may involve the export of proteins of which expression is under the control of RpoN transcription factor.

FEMS7-1055
Physiology / Biochemistry / Molecular Microbiology - Part III

THE IDENTIFICATION OF A NOVEL REGULATORY NETWORK BETWEEN VIRULENCE REGULATOR YMOA AND QUORUM SENSING IN YERSINIA PSEUDOTUBERCULOSIS

A. Slater[1], J. Purves[2], P. Williams[1], S. Atkinson[1]
[1]The University of Nottingham, Centre for Biomolecular Sciences, Nottingham, United Kingdom
[2]The University of Nottingham, Department of Genetics, Leicester, United Kingdom

Backgrounds

Yersinia pseudotuberculosis is an enteropathogen colonising both the mammalian host and the food/water environment. To facilitate this biphasic lifestyle, thermo-dependent regulation, driven by key transcriptional regulators, governs the expression of genes on the 70 KB virulence plasmid (pYV), which is responsible for the production of a Type 3 Secretion system. One of these regulators is the histone-like protein YmoA that represses the expression of T3S-related genes on pYV below 37^{o}C.

Intercellular signaling, usually referred to as quorum sensing, is also known to play a role in the regulation of pYV, and in gram negatives, involves the production and sensing of N-acyl homoserine lactone (AHL) signal molecules through *luxRI* homologues: *ypsR/I* and *ytbR/I*.

Objectives

Considering the relationship between QS and T3S, we sought to investigate whether there is a relationship between the *Y. pseudotuberculosis* quorum sensing systems and YmoA.

Methods

To investigate this relationship at the transcriptional level, the *ymoA* promoter region was fused to the *luxCDABE* operon and introduced as a single copy into the *Y. pseudotuberculosis* chromosome. *ymoA* expression was then examined as a function of temperature and growth phase in four quorum sensing mutants by recording bioluminescence throughout growth at 22^{o}C and 37^{o}C.

Conclusions

The expression of *ymoA* was significantly reduced in the mutant background of all four quorum sensing genes at both 22^{o}C and 37^{o}C suggesting a role for quorum sensing as a positive regulator of *ymoA*, thus confirming a relationship between quorum sensing and as a key regulator of virulence.

FEMS7-0165
Physiology / Biochemistry / Molecular Microbiology - Part III

LANTIBIOTICS AS AN ANTIBIOTIC ALTERNATIVE: CAN WE BYPASS THE NATURAL RESISTANCE SYSTEMS IN HUMAN PATHOGENS?

S. Smits[1]
[1]*Heinrich-Heine Universität Düsseldorf, Institue for biochemistry I, Duesseldorf, Germany*

Backgrounds

Antibiotic resistance is a major focus of medical research throughout the world. Lantibiotics are small antimicrobial peptides possessing high potency and specificity, and thus, are considered to be an excellent and novel candidate as an alternative to antibiotics. Their high activity is reflected by the observation that already 2-4 nM of the lantibiotic nisin is enough to kill a *L. lactis* culture. However, some human pathogenic strains are inherently resistant against these lantibiotics due to the up-regulation of a gene cluster (1). We focused on this gene cluster from the human pathogenic bacteria S*. agalactiae* that confers resistance against nisin. This gene cluster encodes for 4 functional proteins: the membrane-anchored nisin resistance protein *Sa*NSR, the ABC transporter *Sa*NsrFP and the two-component system comprising of *Sa*NsrR and *Sa*NsrK (2).

*Sa*NSR represents a belongs to the C-terminal specific protease superfamily, which cleaves the substrate nisin, thereby, lowering its antimicrobial activity. We solved the high-resolution 2.1 A X-ray structure of *Sa*NSR (3). Using mutagenesis *in vivo* assays and molecular modeling, we were able to identify that *Sa*NSR recognizes exclusively the C-terminus of nisin.
The ABC transporter *Sa*NsrFP belongs to the BceAB superfamily of ABC transporter and has a brought substrate specificity recognizing several different lantibiotics. It removes nisin from the membrane thereby inhibiting nisin mediated membrane pore formation.

Here, we will highlight our latest results towards understanding (I) the exact molecular mechanism of lantibiotic resistance as well (II) dissecting both the individual function of *Sa*NSR and *Sa*NsrFP.

Objectives

Human pathogens
Inhibition

Methods

X-ray crystallography
Growth inhibition
Molecular dynamics and modeling
Protein purification

Conclusions

*Sa*Nsr and *Sa*NsrFP contribute to lantibiotic resistance via two different but very distinct mechanisms. Only when both proteins are present full resistance in human pathogens.

FEMS7-0674
Physiology / Biochemistry / Molecular Microbiology - Part III

INTRA- AND INTERSPECIES GENOMIC DIVERSITY OF CLINICAL ISOLATES OF PHYTOBACTER SPECIES FROM DIFFERENT GEOGRAPHICAL AND TEMPORAL ORIGIN

F. Rezzonico[1], L. Arend[2], H. D'Espindula[3], M. Mira[3], J. Blom[4], J. Pothier[1], M. Pillonetto[3], T. Smits[1]
[1]Research Group Environmental Genomics and Systems Biology- IUNR, Life Sciences and Facility Management, Wädenswil, Switzerland
[2]Molecular Bacteriology Division, Central Public Health Laboratory – State of Paraná, São José dos Pinhais, Brazil
[3]Core for Advanced Molecular Investigation- Graduate Program in Health Sciences, School of Medicine- Pontifícia Universidade Católica do Paraná, Curitiba, Brazil
[4]Bioinformatics and Systems Biology, Justus-Liebig-University, Giessen, Germany

Backgrounds

The genus *Phytobacter* has been found to harbour clinical isolates from a septicaemia outbreak occurring in Brazilian hospitals in 2013. Furthermore, strains previously included in Biotype XII (Brenner, 1984) of the former *Erwinia herbicola-Enterobacter agglomerans* complex (EEC) and isolated in United States during similar outbreaks in the 1970's could now also be assigned to this genus.

Objectives

Studying the intra- and interspecies genomic diversity of *Phytobacter* spp.

Methods

We have sequenced and assembled the genomes of multiple isolates of *P. diazotrophicus* and *P. ursingii*, the two species currently assigned to the genus, and compared them *in silico* using EDGAR 2.0. Genome sequences of two Enterobacteriaceae isolates retrieved in the NCBI database under erroneous species designation were also included in the comparison.

Conclusions

The genus is clearly distinct from other Enterobacteriaceae, with *Kosakonia* being the nearest neighbour. Comparative genomics identified several genus-specific features like the ability of nitrogen fixation from N_2, while at the species level the genetic differences underlying the differential substrate utilization between *P. diazotrophicus* and *P. ursingii* could be confirmed. Analysis of CRISPR arrays showed that Cas genes, only found in one isolate, must have been present in other isolates as well, as remnants of CRISPR arrays were found in their genomes. Presence of plasmids and phages indicate that the diversity may be influenced largely by horizontal gene transfer.

FEMS7-3155
Physiology / Biochemistry / Molecular Microbiology - Part III

BACILLUS POLYFERMENTICUS B28 EXTRACT SUPPRESSES MELANOCYTE DIFFERENTIATION BY ATTENUATING THE WNT/ B-CATENIN PATHWAY

Y. Son[1], H.E. Jin[1], M.S. Kwak[1], M.H. Sung[1], S. Oh[1]
[1]Kookmin University, Bio and Fermentation Convergence Technology, Seoul, Republic of Korea

Backgrounds

The Wnt/b-catenin pathway regulates neural crest-derived melanocyte development and is thus a potential target for the development of whitening agents.
Bacillus polyfermenticus, known as a probiotic bacterium, has been used clinically for the treatment of various intestinal disorders. It also exhibits a number of physiological activities, such as antmicrobial activity, cholesterol-reducing activity, stimulation of IgG production, and an antiproliferative effect on Caco-2 colon cancer cells.

Objectives

The target of this study is to find the the extract of *Bacillus polyfermenticus* B28 capable of regulating Wnt/β-catenin signaling and how to inhibit melanocyte differentiation.

Methods

We used HEK293-FL reporter cells that were stably transfected with a human Frizzled-1 (*hFz-1*) expression plasmid and a synthetic b-catenin/Tcf-dependent luciferase reporter plasmid to screen a bacterial extract that inhibits Wnt/b-catenin signaling.
To prepare BPE, *B. polyfermenticus* B28 were grown in Luria Bertani (LB) broth medium for 18 h and then pelleted by centrifugation. The pellet was solubilized in 20 mM potassium phosphate buffer (pH 7.4) solution, sonicated, and homogenized by microfluidizer (Young Jin Corporation). BPE was prepared by centrifugation of the homogenate using a high speed centrifuge (Hanil Science Inc.) at 12,000 rpm.

Conclusions

B. polyfermenticus B28 extract (BPE) suppressed β-catenin response transcription (CRT), which was activated by Wnt3a-conditioned medium (Wnt3a-CM) by downregulating intracellular β-catenin in HEK293 reporter cells. In addition, BPE decreased the levels of intracellular β-catenin, microphthalmia-associated transcription factor (MITF), and tyrosinase in B16 melanoma cells, thereby reducing intracellular melanin production. Our findings indicate that BPE may have potential as a whitening agent for use in cosmetics and in the medical treatment of hyperpigmentation disorders.

FEMS7-0989
Physiology / Biochemistry / Molecular Microbiology - Part III

ROLE OF MER IN THE TRANSPORT OF TOXIC METALS IN ESCHERICHIA COLI

Y. Sone[1], S. Uraguchi[1], R. Nakamura[1], Y. Takanezawa[1], M. Kiyono[1]
[1]Kitasato University, School of Pharmacy, Tokyo, Japan

Backgrounds

The characteristics of bacteria take up mercury into cells via Mer superfamily, i.e., MerC, MerE, MerF, and MerT, which transport mercuric ions into the cytoplasm, have been applied in engineering of bioreactor used for mercurial bioremediation. In all these proteins, one pair of cysteine residues is predicted to reside within the inner membrane. However there is no direct experimental evidence concerning bacterial transport of toxic metals until recently.

Objectives

Our objective was to clarify individual role of MerC, MerE, MerF, or MerT and potential in transport of toxic metals.

Methods

The toxic metals uptake capacities of *E. coli* cells that carried control vector or *mer* recombinants were examined. The cells were suspended in LB broth containing 5 μM $^{14}CH_3Hg^+$, 5 μM $C_6H_5Hg^+$, 10 μM Hg^{2+}, 300 μM Cd^{2+}, 10 μM AsO_2^-, 50 μM $HAsO_4^{2-}$, or 10 μM CrO_4^{2-} and incubated for various time at 37°C, respectively. The total metal of the harvested cells were measured using a liquid scintillation spectrometer ($^{14}CH_3Hg^+$), atomic absorption spectrometry analyzer ($C_6H_5Hg^+$ or Hg^{2+}) or inductively coupled plasma optical emission spectrometry analyzer (AsO_2^-, $HAsO_4^{2-}$ or CrO_4^{2-}), respectively.

Conclusions

The cells that expressed MerC, MerE, MerF, or MerT accumulated significantly more CH_3Hg^+, $C_6H_5Hg^+$, Hg^{2+}, AsO_2^-, or $HAsO_4^{2-}$ than control. MerC recombinants accumulated significantly more CrO_4^{2-} than control. Consequently, we demonstrated that MerC, MerE, MerF, and MerT are broad-spectrum toxic metals transporters. Our results suggested that all Mer transporters can be used for designing toxic metals bioremediation systems, moreover, MerC is the most efficient tool.

FEMS7-1184
Physiology / Biochemistry / Molecular Microbiology - Part III

CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF MITOGEN ACTIVATED PROTEIN KINASES IN THE DIMORPHIC FISSION YEAST SCHIZOSACCHAROMYCES JAPONICUS

T. Soto[1], P. Soluyanova[1], E. Gómez-Gil[1], B. Vázquez-Marín[1], M. Madrid[1], J. Vicente-Soler[1], M. Gacto[1], J. Cansado[1], A. Franco[1]
[1]Univesity of Murcia, Department of Genetics and Microbiology, MURCIA, Spain

Backgrounds

Mitogen-activated protein kinase (MAPK) cascades are universal signal transduction modules that play crucial roles in eukaryotic cells. In contrast to the well studied fission yeast *Schizosaccharomyces pombe*, a second species within the genus named *Schizosaccharomyces japonicus* shows a larger cell size and specific features including semi-open mitosis, light response and dimorphism.

Objectives

Like in other dimorphic fungi, this simple eukaryote may provide an attractive and suitable model to comparatively analyze MAP kinase signaling mechanisms and their role during the stress response and the transition from yeast to hyphal growth.

Methods

Here we show that Sty1^{Sj}, a p38-type MAPK, is involved in the adaptive cellular responses to stress and negatively regulates mycelial growth. Pmk1^{Sj}, an ERK-type MAPK, also responds to multiple stresses, but positively regulates cell branching and mycelial expansion. Remarkably, while Sty1 amino-acid sequence and structure is strongly conserved in both fission yeast species, Pmk1^{Sj} lacks a 24-amino acid motif within its N-terminal ATP-binding domain which is present and critical for activation in all eukaryotic MAP kinases studied so far. Moreover, Pmk1^{Sj} is totally functional in *S. pombe* cells, suggesting the existence of other modifications within Pmk1^{Sj} structure to bypass the need of a canonical ATP-binding domain domain for MAPK activation.

Conclusions

Our results suggest that in *S. japonicus* both Sty1^{Sj} and Pmk1^{Sj} positively regulate the cellular response to environmental changes, but play opposing roles during the regulation of the dimorphic transition.

FEMS7-1940
Physiology / Biochemistry / Molecular Microbiology - Part III

PHYLOGROUP RECLASSIFICATION OF ESCHERICHIA COLI ISOLATES BY THE REVISED EXTENDED QUADRUPLEX PHYLOTYPING METHOD

M. Starčič Erjavec[1], *D. Žgur-Bertok*[1]
[1]*University of Ljubljana - Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia*

Backgrounds

Clermont et al. in 2000 published a simple triplex PCR based phylotyping method. Based on newly available genome data Clermont et al. in 2013 proposed a new revised method of PCR phylotyping, the extended quadruplex PCR that was validated on two collections of human fecal isolates.

Objectives

The aim of our study was to broaden the validation of the study with additional *E. coli* isolates: human skin and soft-tissue infection *E. coli* isolates (SSTIEC), fecal *E. coli* isolates from healthy humans (hFEC), brown bear fecal *E. coli* (bFEC) and cattle fecal *E. coli* (cFEC).

Methods

The *E. coli* strains investigated in this study (102 SSTIEC, 90 hFEC, 86 bFEC and 89 cFEC) were gathered and partially characterized in our previous studies. In this study all the investigated strains were PCR phylotyped with the new extended quadruplex PCR method as published by Clermont et al. in 2013.

Conclusions

Our study showed that the most pronounced changes in the distribution of strains among phylogenetic groups were within the D group strains, as when employing the extended quadruplex method, the majority had to be re-placed into other phylogenetic groups. A considerable number of re-placements were also observed among A group strains, while less for the B1 and B2 groups. We conclude that the extended quadruplex PCR method is indeed a superior method for PCR phylotyping however, as some strains still (11% of strains in the case of cattle *E. coli*) remained unclassified, a revision of the revised method is needed.

FEMS7-3089
Physiology / Biochemistry / Molecular Microbiology - Part III

IMPACT OF GLUTATHIONE METABOLISM ON ZINC HOMEOSTASIS IN SACCHAROMYCES CEREVISIAE

M.G. Steiger[1], A. Patzschke[1], C. Holz[2], C. Lang[2], T. Causon[3], S. Hann[3], D. Mattanovich[4], M. Sauer[4]
[1]Austrian Centre of Industrial Biotechnology, Systems Biology & Microbial Cell Engineering, Vienna, Austria
[2]Organobalance GmbH, Organobalance, Berlin, Germany
[3]University of Natural Resources and Life Sciences- Vienna BOKU, Department of Chemistry, Vienna, Austria
[4]University of Natural Resources and Life Sciences- Vienna BOKU, Department of Biotechnology, Vienna, Austria

Backgrounds

Zinc is a crucial mineral for all organisms as it is an essential cofactor for the proper function of a plethora of proteins and depletion of zinc causes oxidative stress. Therefore, it is highly important to understand how the cell can maintain and control metal homeostasis. Glutathione is the major redox buffering agent in the cell and therefore important to mitigate adverse effects of oxidative stress. In mammalian cells zinc deficiency is accompanied by a glutathione depletion. In the yeast *Saccharomyces cerevisiae* the opposite effect is observed: under low zinc conditions an elevated glutathione concentration is found. The main regulator to overcome zinc deficiency is Zap1.

Objectives

In this work, we set out to characterize the impact of glutathione and its derivatives on zinc homeostasis and if the transcriptional regulator Zap1 is involved in this cross talk.

Methods

Total zinc concentrations (ICP-MS) and free available zinc pools (fluorescence microscopy) were analysed in *S. cerevisiae* strains differing in their overall glutathione content. Furthermore, cellular phytochelatin levels were measured by LC-MS/MS. Deletion strains of Zap1 were generated and their glutathione content determined.

Conclusions

We show that Zap1p is not involved in a glutathione accumulation phenotype. Furthermore, we found that in glutathione accumulating strains also the metal ion binding phytochelatin-2, which is an oligomer of glutathione is accumulated. This increased phytochelatin concentration correlates with a lower free zinc level in the vacuole. Our results suggest that phytochelatin is important for zinc buffering in *S. cerevisiae* and thus explains how zinc homeostasis is connected with glutathione metabolism.

FEMS7-0517
Physiology / Biochemistry / Molecular Microbiology - Part III

ADAPTING TO COMPETITIVE INHIBITION IN THE COMMUNITY CONTEXT: A TALE OF TWO SULFATE-REDUCING MICROBES

M. Stoeva[1], M. Mehta[1], R. Rushton-Green[2], L. Tran[1], J. Coates[1]
[1]UC Berkeley, Plant and Microbial Biology, Berkeley, USA
[2]University of Otago, Department of Microbiology and Immunology, Dunedin, New Zealand

Backgrounds

Inhibition of sulfate-reducing microorganisms (SRM) is of interest owing to the human-health and economic costs of sulfide, the byproduct of microbial sulfate-reduction. Perchlorate is a potent and specific inhibitor of SRM. For a complete understanding of the stability of inhibition, we are exploring mechanisms by which SRM evolve resistance to perchlorate. Previous work has shown that model SRM, *Desulfovibrio alaskensis g20* can adapt to 100mM perchlorate via a single nucleotide polymorphism (SNP) in the sulfate adenylyltransferase (*sat*).

Objectives

To further this research, we aim to understand adaptation to perchlorate across different SRM, as well as in a community context.

Methods

Sulfidogenic communities were transferred in increasing concentrations of perchlorate and compared to control communities in terms of community structure, frequency of the *sat* SNP and presence/frequency of novel mutations.

Conclusions

Results indicate that the *sat* is a target for adaptation in pure culture significantly more frequently than in a community, likely owing to the relatively higher cost of this mutation. In a community setting, a null mutation of a periplasmic histidine kinase (dde_0320) dominates. The phenotype of this mutation was confirmed using a transposon mutant of dde_0320. Currently, an RNAseq experiment is underway, to identify regulatory targets of dde_0320. *Desulfovibrio singaporenus MKS*, a SRM native to the sulfidogenic community, is also capable of adaptation to 100mM perchlorate, albeit by a different mechanism: *D. singaporenus* exhibits SNPs in transporter proteins. Efforts are currently underway to characterize these mutants. Thus, this works aims to describe ecologically-relevant adaptive mechanisms across diverse SRM against competitive inhibition.

FEMS7-2178
Physiology / Biochemistry / Molecular Microbiology - Part III

CHARACTERIZATION OF B-D-MANNOSIDASE FROM BACILLUS SP

W. Sukhumsirichart[1], W. Seesom[1], T. Sakamoto[2]
[1]Srinakharinwirot University, Department of Biochemistry- Faculty of Medicine, Bangkok, Thailand
[2]Osaka Prefecture University, Department of Applied Life Sciences, Osaka, Japan

Backgrounds

β-D-mannosidase (EC 3.2.1.25) are glycoside hydrolase enzymes that cleave β-1,4-mannosidic-linked mannosides from the non-reducing end of various β-1,4-linked mannooligosaccharides. It is essential for the complete hydrolysis of mannan.

Objectives

To screen for β-D-mannosidase -producing microorganisms and to observe the characteristics of the extracted crude mannosidase enzyme.

Methods

A total of 299 microorganisms were screened on minimum medium containing locust bean gum at 37°C, and the species of the highest mannosidase producing microorganism was identified by 16S rDNA sequence analysis. Temperature and pH optimum and stability of the crude enzyme were examined.

Conclusions

Analysis of the 16S rDNA sequence revealed that the highest mannosidase producing microorganism was *Bacillus* sp. The activity of the crude enzyme was 0.8 unit/ml using 4-nitrophenyl β-D-monopyranoside as a substrate. The optimal pH and temperature for enzyme activity were observed to be 7.0 and 50°C, respectively. The enzyme was stable in pH ranges from 5 to 9, after 16 h of incubation at 4°C and stable up to 40°C for 1 h, and more than 80 % of initial activity remained. This exo-β-1,4-mannosidase can enzymatically release D-mannose from galactomannan, glucomannan and mannan that can be used for the production of bioethanol and other biochemical from biomass.

FEMS7-0790
Physiology / Biochemistry / Molecular Microbiology - Part III

COMPLETE GENOME SEQUENCE OF LACTOCOCCUS LACTIS G50 AND PILIN LOCUS STRUCTURE

C. Suzuki[1], K. Nakano[2], N. Moriya[1], M. Minami[2], M. Shinzato[2], M. Shimoji[2], N. Ashimine[2], A. Shiroma[2], S. Ohki[2], T. Nakanishi[2], H. Tamotsu[2], K. Teruya[2], K. Satou[2], H. Kimoto-Nira[1], M. Kobayashi[1], T. Hagi[1], M. Nomura[1], T. Hirano[2]
[1]National Agriculture and Food Research Organization, Institute of Livestock and Grassland Science, Tsukuba, Japan
[2]Okinawa Institute of Advanced Sciences, Research and Development Division, Uruma- Okinawa, Japan

Backgrounds

Lactococcus lactis subsp. *lactis* G50 isolated from Napiergrass has an ability to induce the production of cytokines (IL-12, IL-6, and TNF-α) in macrophage-like cell line J774.1. The stimulus required for TNF-α induction is heat sensitive, because heat-treated G50 loses the activity to induce TNF-α.

Objectives

To identify potential genetic determinants giving immunostimulatory activity to G50, complete genome sequence was obtained and analyzed by the single-molecule real-time (SMRT) technology.

Methods

The genomic DNA was purified at the early log phase, followed by 20-kb library construction for P6-C4 chemistry with shearing. Size selection was not performed. Two SMRT cells (each 240-min movies) were sequenced using the PacBio RS II platform (Pacific Biosciences, Menlo Park, CA). *De novo* assembly was performed using the hierarchical genome assembly process version 2. The G50 genome has been annotated with DDBJ MiGAP. Protein localization was predicted by PSORTb.

Conclusions

A single circular contig representing one chromosome (2,346,663 bp, G+C content of 35.03%, and 925× coverage) was obtained. No plasmids were detected either by assembly or gel electrophoresis. The genome of G50 was similar to that of *L. lactis* KF147 (query cover 89%, identity 99%). Using PSORTb, 57 proteins in 2621 ORF in the genome were deduced as either extracellular or attached to the cell wall, including a set of pilus proteins, YhgD, YhgE, and YhhB. The pilin tip protein YhgD is unique in that the C-terminal half of YhgD is strain specific. The pilin locu of lactococci were compared and discussed as a candidate of immunostimulating factor.

FEMS7-2098
Physiology / Biochemistry / Molecular Microbiology - Part III

EPIGENETIC REGULATION OF GENE EXPRESSION DURING GIARDIA INTESTINALIS ENCYSTATION

E. Einarsson[1], U. Ribacke[2], L. Rojas[1], S. Svärd[1]
[1]Uppsala University, Department of Cell and Molecular Biology, Uppsala, Sweden
[2]Karolinska Institutet, Department of Microbiology- Tumour and Cell Biology, Stockholm, Sweden

Backgrounds

Infectious cysts of the intestinal protozoan parasite *Giardia intestinalis* are formed through the process of encystation, making this cellular differentiation crucial for transmission and survival. Other protozoan parasites like the malaria parasite *Plasmodium falciparum* have been shown to regulate differentiation between different life-cycle stages on the epigenetic level.

Objectives

To further study the molecular details of *Giardia* encystation, focusing on epigenetic regulation of gene expression.

Methods

We used our newly developed *in vitro* encystation protocol to generate differentiating cells from each 1.5 hrs of the 30 hrs encystation process. Transcriptional changes during the entire differentiation from trophozoites to cysts were studied using RNA sequencing (RNA-seq), genomic methylation detection and chromatin immunoprecipitation.

Conclusions

A high level of periodicity was observed for up- and down-regulated genes, both at the level of the entire transcriptome and putative regulators. The largest transcriptional changes were seen in the late phase of encystation with the majority of the highly up-regulated genes encoding hypothetical proteins and variant specific surface proteins (VSPs). The transcriptomic analyses were complemented by analyses of the methylation and histone modification status of selected encystation-regulated genes and these analyses suggest an important role of epigenetic regulation of gene expression during *G. intestinalis* encystation. Our results underline the importance of epigenetic regulation of gene expression during antigenic variation and differentiation in protozoan parasites.

FEMS7-0065
Physiology / Biochemistry / Molecular Microbiology - Part III

QUANTITATIVE ANALYSES OF DNA AND RNA IN ENLARGED SPHEROPLASTS OF THE BACTERIUM LELLIOTTIA AMNIGENA.

S. Takahashi[1], *H. Nishida*[1]
[1]*Toyama Prefectural University, Biological Engineering, Imizu, Japan*

Backgrounds

Generally, bacterial spheroplasts do not divide, but they can grow and enlarge if placed in a broth containing a peptidoglycan synthesis inhibitor and high concentrations of salt. Spheroplasts of *Lelliottia amnigena*, from the family Enterobacteriaceae, enlarged up to >30 μm in diameter at the maximum (Takahashi & Nishida, 2016, Bull Toyama Pref Univ, 26, 27-30). Total DNA in spheroplasts increased during enlargement, suggesting that DNA replicates in spheroplasts (Takahashi & Nishida, 2015, J Gen Appl Microbiol, 61, 14-17). In addition, we compared RNA expression between early-growth spheroplasts and enlarged spheroplasts (Takahashi et al., 2016, AIMS Microbiol, 2, 152-189).

Objectives

In this study, we quantified DNA from a single spheroplast and RNA from normally-dividing cells, early-growth and enlarged spheroplasts, and filamentous cells generated from spheroplasts.

Methods

We selected spheroplasts with an inner membrane diameter of 9-15 μm and isolated a single cell using a micromanipulator at 24 h, 48 h, 72 h, and 96 h of growth. Concentrations of DNA were quantified using qPCR. RNA was isolated from normally-dividing cells, early-growth and enlarged spheroplasts, and filamentous cells. RT-qPCR was performed for the following 10 genes: cell division-related genes *ftsA* and *ftsZ*; DNA replication-related genes *hupA*, *topA*, and *recA*; stress response-related genes *clpB*, *pspA*, and *spy*; and cell surface-related genes *ompA* and *pbp1b*.

Conclusions

The amount of DNA in a single spheroplast did not increase after 24 h of growth. It suggests that the DNA replication ceased in enlarged spheroplasts after 24h of growth. Interestingly, the outer membrane continued to grow after 24h of growth. RNA expressions were different between enlarged spheroplasts and filamentous cells. For example, *clpB* and *ftsZ* were upregulated during enlargement, whereas these two genes were downregulated during filamentation. Conversely, *hupA* was downregulated during enlargement, but upregulated during filamentation.

FEMS7-0239
Physiology / Biochemistry / Molecular Microbiology - Part III

LITR, A MERR-FAMILY TRANSCRIPTIONAL REGULATOR OF BACILLUS MEGATERIUM: ITS ROLE AND FUNCTION IN LIGHT-INDUCIBLE CAROTENOID PRODUCTION

H. Takano[1], K. Mise[1], K. Hagiwara[1], N. Hirata[1], S. Watanabe[1], H. Takano[1], K. Ueda[1]
[1]Nihon University, College of Bioresource Sciences, Fujisawa- Kanagawa, Japan

Backgrounds

Carotenoid production in certain non-phototropic bacteria protects cells from photo-oxidants and occurs in a light-dependent manner. The LitR/CarH family of transcriptional regulators acts as light-dependent, negative regulators of carotenoid genes in gram-negative bacteria such as *Thermus thermophilus* and *Myxococcus xanthus*. LitR repressor activity is controlled by adenosyl B12, a light-sensitive chromophore. However, biochemical characterization of the LitR/CarH family has been confined to gram-negative bacteria.

Objectives

We therefore investigated the function and role of the LitR homolog in the gram-positive, soil bacterium, *Bacillus megaterium* QM B1551.

Methods

B. megaterium wild type produced carotenoid in a light-dependent manner. However, the *litR* null mutant exhibited constitutive, light-independent carotenoid production, while the *litR*-complemented strain produced carotenoid in a light-dependent manner. Transcriptional analysis revealed that the expression of carotenoid biosynthesis genes is light-dependently regulated in the wild type strain; however, transcription of *litR* in the mutant was constitutive, indicating that LitR serves as a light-sensitive repressor that controls the expression of carotenoid genes. In concordance with these results, the LitR recombinant protein in complex with adenosyl B12 bound to the carotenoid promoter region in a specific and light-sensitive manner. *In vitro* transcriptional run-off assay demonstrated that the mixture of LitR and RNA polymerase light-dependently generates a transcript of the *crt* gene.

Conclusions

Overall, our results indicate that LitR of *B. megaterium* functions as an adenosyl B12-based, light-sensitive regulator.

FEMS7-0914
Physiology / Biochemistry / Molecular Microbiology - Part III

REGULATION OF GRATA TOXIN-ANTITOXIN SYSTEM OF PSEUDOMONAS PUTIDA

H. Tamman[1], *A. Ainelo*[2], *R. Hõrak*[2]
[1]*Free University of Brussels ULB, Biologie Structurale et Biophysique, Gosselies, Belgium*
[2]*University of Tartu- The Institute of Molecular and Cell Biology, Chair of Genetics, Tartu, Estonia*

Backgrounds

Type II toxin-antitoxin (TA) systems consist of two proteins: a stable toxin that damages its host bacterium and an unstable antitoxin, able to inhibit the toxin. Many TA loci are regulated by conditional cooperativity, where the toxin, depending on its abundance, can act either as a co-repressor or de-repressor of transcription.
The toxin of GraTA system has been shown to decrease growth rate and influence stress tolerance of *Pseudomonas putida*. The quite stable antitoxin GraA inhibits the toxin GraT from causing cold-sensitive ribosome biogenesis defect by binding it into a complex.

Objectives

The current work set out to study the regulation of the GraTA system.

Methods

The transcriptional regulation of the *graTA* locus was studied by using a transcriptional fusion of *graTA* promoter and *lacZ* reporter gene both in *P. putida* and *E. coli*. The abundance of GraTA proteins was determined by Western blotting.

Conclusions

Differently from many other TA systems, *graTA* expression is not regulated by conditional cooperativity. We show that the *graTA* operon is autorepressed by antitoxin only, whereas the toxin is a de-repressor. Data also indicate that although the *graT* gene precedes *graA*, translation of the antitoxin is more efficient than the toxin's. This mechanism guarantees that GraA is produced in higher amounts than GraT and thus ensures the silencing of the toxin. Unequal translation of TA genes is likely to also play a role in the regulation of other TA operons with a similar gene order.

FEMS7-1929
Physiology / Biochemistry / Molecular Microbiology - Part III

IDENTIFICATION OF UDP-GLUCOSE 4-EPIMERASE (GALE) GENE ISOLATING FROM THE ORAL METAGENOMIC DNA CONFERS RESISTANCE TO QUATERNARY AMMONIUM COMPOUNDS (QACS)

S. Tansirichaiya[1], L.J. Reynolds[1], G. Cristarella[1], A.P. Roberts[1]
[1]UCL Eastman Dental Institute, Microbial Disease, London, United Kingdom

Backgrounds

Overexposing and misusing of antimicrobial compounds results in bacteria developing resistance. The human oral cavity is one environment exposed to these compounds through foods and oral hygiene products.

Objectives

To identify and characterise antimicrobial resistance genes from the human oral metagenome.

Methods

We have adopted a metagenomic approach to screen for resistance genes from both culturable and unculturable bacteria in the human oral cavity. An oral metagenomic library, constructed in *Escherichia coli* using pCC1BAC vector, was screened against several antimicrobials.

Conclusions

Out of 12,277 clones screened, one clone was found to have resistance against two commonly used antiseptics, cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC). Analysis of the plasmid from this clone showed that it contained a 17.1 kb insert, and the resistance was conferred by a UDP-4 glucose-epimerase (*galE*) gene homologous to one from *Veillonella parvula*. The product of *galE* is involved in LPS production. Analysis of the *E. coli* host showed the cell surface charge was more positive in the presence of *galE*, which could reduce the binding of these positively charges antiseptics, like CTAB and CPC, to the bacteria.

This is the first time that *galE* has been shown to be responsible for resistance against QACs.

FEMS7-0295
Physiology / Biochemistry / Molecular Microbiology - Part III

A FAMILY OF SRNAS PLAYS A ROLE IN THE RESPONSE OF LISTERIA MONOCYTOGENES TO HEME TOXICITY

P. T. dos Santos[1], D. Sabharwal[1], P. Menendez-Gil[1], E.M. Sternkopf Lillebæk[1], B. H. Kallipolitis[1]
[1]University of Southern Denmark, Biochemistry and Molecular Biology, Odense M, Denmark

Backgrounds

At present, over 200 putative small non-coding regulatory RNAs (sRNAs) have been identified in *Listeria monocytogenes*. Interesting, several sRNAs have been identified as being induced in human blood. *L. monocytogene*s has the ability to lyse erythrocytes, remove heme from hemoglobin and liberate Fe^{2+} from heme. Even though iron is essential for life, it is highly toxic under aerobic conditions, as it reacts with oxygen species forming free radicals. Thus, *L. monocytogenes* needs to find a way to overpass the unfavorable conditions it encounters in the presence of excess heme.

Objectives

To understand why some sRNAs are highly induced in human blood, we hypothesized that this induction could be caused by high levels of heme in this environment. Therefore, the aim was to investigate the role of heme-induced sRNAs in response to excess heme and look for their putative targets in *L. monocytogenes.*

Methods

Wild-type cells were subjected to increasing concentrations of hemin and sRNAs levels were determined via Northen Blot analysis. To verify their role in the prevention of heme toxicity, growth of a strain lacking sRNAs was compared to the wild-type strain. Finally, a search was performed to identify possible targets of the sRNAs under hemin stress.

Conclusions

A family of sRNAs was greatly induced by hemin, and a strain lacking the sRNAs showed impaired growth in the presence of hemin, suggesting a fine-tuning role for these sRNAs in the prevention of heme toxicity. Putative target genes were identified and the mechanisms underlying the regulation by the sRNAs are under investigation.

FEMS7-2065
Physiology / Biochemistry / Molecular Microbiology - Part III

WHOLE-GENOME SEQUENCING UNVEILS A POTENTIALLY NEW PSEUDOMONAS SPECIES ASSOCIATED WITH TN6372, A NOVEL BLAVIM-2-TRANSPOSABLE ELEMENT

J. Botelho[1], *F. Grosso*[1], *L. Peixe*[1]
[1]*UCIBIO/REQUIMTE- Laboratório de Microbiologia- Faculdade de Farmácia- Universidade do Porto- Porto- Portugal, Microbiology, Porto, Portugal*

Backgrounds

The *Pseudomonas putida* group represents a diverse cluster of opportunistic pathogens, such as *Pseudomonas putida*, *Pseudomonas monteilii* and *Pseudomonas mosselii*. Due to their resemblance, species identification is somehow challenging. Several studies reported the need to re-examine the differentiation of species within *P. putida* phylogenetic group.

Objectives

Here, we characterize the genetic environment of a carbapenemase gene in a clinical isolate belonging to a potentially new *Pseudomonas* species.

Methods

FFUP_PS_41 isolate, recovered from endotracheal tube secretions of a Neonatal/Pediatric Intensive Care unit patient with pneumonia, exhibited an extensively-drug resistant phenotype (only susceptible to colistin). Species identification was assessed by VITEK-2 system and confirmed by multi-locus sequence analysis (*rpoD*, *gyrB*, *rpoB* and 16S rRNA genes) and average nucleotide identity (ANI) analysis. The complete nucleotide sequence (6,523,850 bp; 62,2% GC content) was achieved by Illumina HiSeq sequencing platform and *de novo* assembly by SPAdes. Although FFUP_PS_41 isolate was identified as *P. putida* by VITEK-2, it displayed an ANI value <95% (cut-off for species identification) when compared with the complete genome of type strains belonging to the *P. putida* group. A strong correlation was observed with MLSA (<97% cut-off), suggesting that FFUP_PS_41 represents a new species, related to *P. putida* group. bla_{VIM-2} was associated with In103, a class 1 integron co-harboring aminoglycoside resistance genes (*aacA7* and *aacA4*). In103 was inserted in a novel putative transposon, hereby named Tn*6372*.

Conclusions

FFUP_PS_41 isolate most likely represents a new species, belonging to *P. putida* phylogeny group. The new transposable element highlights the ongoing process of bla_{VIM-2} dissemination throughout different species.

FEMS7-2110
Physiology / Biochemistry / Molecular Microbiology - Part III

COMPLETE NUCLEOTIDE SEQUENCE OF PJB58, A BLAVIM-2-HARBORING PLASMID FROM PSEUDOMONAS AERUGINOSA

J. Botelho[1], F. Grosso[1], A. Mabrouk[2], L. Peixe[1]
[1]UCIBIO/REQUIMTE- Laboratório de Microbiologia- Faculdade de Farmácia- Universidade do Porto- Porto- Portugal, Microbiology, Porto, Portugal
[2]Laboratories UR12ES02-
The National Bone Marrow Transplant Centre- Tunis- Tunisia- University of Carthage- Faculty of Scie nces of Bizerte- Tunis- Tunisia, UR12ES02-The National Bone Marrow Transplant Centre, Tunis, Tunisia

Backgrounds

VIM-2 represents one of the most widespread carbapenemases among *Pseudomonas aeruginosa*. *bla*$_{VIM-2}$-carrying In58 integron has been mainly linked to a chromosomal location in different bacterial species, including *P. aeruginosa*.

Objectives

Here, we characterize the genetic environment of an In58-carrying plasmid.

Methods

Complete nucleotide sequence of plasmid pJB58, recovered from *P. aeruginosa* ST282 clinical isolate exhibiting a multidrug-resistant phenotype, was achieved by Illumina HiSeq and *de novo* assembly by SPAdes. Plasmid transferability was evaluated by conjugation. pJB58 (32,207 bp, 58,6% GC content) was related to *bla*$_{VIM-1}$-harboring plasmids pPC9, from *Pseudomonas putida* HB3267 strain from France (88% query cover and 99% identity) and pAMBL2, from *P. aeruginosa* in Spain (44% query cover and 99% identity), including the partitioning and stable inheritance system (*rep*, *kfrA* and *vapBC* toxin-antitoxin system). Replicase protein belongs to Rep-3 superfamily and shared 100% homology with the ones from pPC9 and pAMBL2. pJB58 lacked the complete machinery for self-conjugation, justifying the failure of conjugation. Yet, it might be mobilizable by a helper plasmid, since it carried a relaxase gene (*trwC*/*traI*), a putative *oriT* site and a type-IV coupling protein gene (*trwB*/*traJ*). Similar findings were observed for pPC9. A Tn*402*-like transposon comprising the In58 integron, harboring *bla*$_{VIM-2}$ and aminoglycoside resistance genes (*aacA7*, *aacC1* and *aacA4*), and IS*6100* was identified. A Tn*5393*c-like transposon harboring streptomycin resistance genes was also reported.

Conclusions

Complete nucleotide sequence of plasmid pJB58 from *P. aeruginosa* revealed the *bla*$_{VIM-2}$-harboring In58 integron in a putative mobilizable plasmid. These findings provide new insights on the mobile genetic elements associated with VIM-2 dissemination.

FEMS7-2430
Physiology / Biochemistry / Molecular Microbiology - Part III

FIRST PHYSIOLOGICAL STUDY ON NOX EMISSIONS IN HALOARCHAEA: ARE THEY INVOLVED IN CLIMATE CHANGE?

J. Torregrosa-Crespo[1], L. L. Bergaust[2], C. Pire Galiana[1], L. R. Bakken[3], R.M. Martínez-Espinosa[1]
[1]Universidad de Alicante, Agrochemistry and Biochemistry, ALICANTE, Spain
[2]Faculty of Chemistry- Biotechnology and Food Science. Norwegian University of Life Sciences. Foug nerbakken 3 - 1430- Ås- Norway., Department of Chemistry- Biotechnology and Food Science., Ås, Norway
[3]Faculty of Environmental Sciences and Natural Resource Management. Norwegian University of Life Sciences. Fougnerbakken 3 - 1430- Ås- Norway., Department of Environmental Sciences., Ås, Norway

Backgrounds

Haloarchaea are extremophiles inhabiting saline and hypersaline media such as salt lagoons and salty ponds. Knowledge about denitrification in these microorganisms and in the Archaea domain in general is scarce. The few studies executed so far have dealt with biochemical aspects of the pathway[1], and to date there are no reports of physiological experiments addressing the release of gaseous nitrogen oxides (NO_x) by this group of microorganisms.

Objectives

This work is the first comprehensive study on NO_x emissions from Archaea. Three Haloarchaea strains, *Haloferax mediterranei, Haloferax denitrificans* and *Haloferax volcanii* were selected as model organisms and characterized with respect to denitrification phenotype.

Methods

A semi-automatic incubation system was used to monitor the kinetics of all relevant gases (CO_2, O_2, NO, N_2O, N_2)[2] during transitions from aerobic respiration to denitrification in batch cultures with low initial O_2 (1 vol% in headspace) and either nitrate or nitrite.

Conclusions

The results confirm that *Hfx. mediterranei* and *Hfx. denitrificans* are complete denitrifiers, producing N_2 as final product. All the available nitrate (5 mM) was recovered as N_2 with transient accumulation of nM concentrations of NO and N_2O. In contrast, *Haloferax volcanii* is a partial denitrifier, unable to reduce nitrate and N_2O, thus generating N_2O as final product in nitrite supplemented medium. Considering the large areas that haloarchaea inhabit on Earth, the results suggest that these extremophiles could represent a new group of microorganisms of relevance to NO_x emissions and climate change. However, complete denitrifiers could be used in wastewater treatments due to their ability to reduce NO_3^-, NO_2^- to N_2 (inert gas).

References

[1] Torregrosa-Crespo J *et al.* 2016. Adv Microb Physiol 68: 41-85.

[2] Molstad L *et al*. 2007. J Microbiol Meth 71: 202-211.

Acknowledgements

This work was funded by research grant from the MINECO Spain (CTM2013-43147-R), Generalitat Valenciana (ACIF2016/077) and European Molecular Biology Organization (ASTF 331-2016).

FEMS7-2450
Physiology / Biochemistry / Molecular Microbiology - Part III

CONTROL ON THE COMPLEX PSEUDOMONAS AERUGINOSA RIBONUCLEOTIDE REDUCTION NETWORK BY THE ALGZR TWO-COMPONENT SYSTEM

E. Torrents[1], *L. Pedraz*[1], *A. Crespo*[1]
[1]*Insitute for Bioengineering of Catalonia IBEC, Bacterial Infections and antimicrobial therapies, Barcelona, Spain*

Backgrounds

DNA synthesis is one of the central processes of life, required for any living cell. The Ribonucleotide Reductase (RNR) family include the only enzymes able to catalyse the reduction of the ribonucleotides to deoxyribonucleotides, the building blocks of the DNA. RNRs comprises three classes of enzymes (class I, class II and class III); although eukaryotic cells codify only class I, bacteria can codify any combination of classes.

In the opportunistic pathogen *Pseudomonas aeruginosa,* we find all three classes codified in the same genome. It is believed that under the different situations this bacterium encounters, in planktonic growth and in biofilms, under free growth and under infections conditions, all three RNR classes are controlled by complicated regulation systems and play different roles.

Objectives

Understand the biological role of AlgR to fine regulate the transcription of the different ribonucleotide reductase genes in *Pseudomonas aeruginosa*.

Methods

In this work, we demonstrate the fine regulation of class I and class II RNR in *P. aeruginosa* by the AlgZR system by analysing their transcriptional regulation depending on different phosphorylation AlgR state as well as their specific interaction to the class Ia and II RNR promoter regions by EMSA.

Conclusions

We investigated how the AlgZR system helps to finely tune up the expression of class I and II RNR classes to adapt to different growing conditions and environmental situations, intertwining for the first time these two regulation networks.

FEMS7-1987
Physiology / Biochemistry / Molecular Microbiology - Part III

BACILLUS SUBTILIS DISA AND RADA COOPERATE WITH HOLLIDAY JUNCTION PROCESSING PROTEINS IN THE RESCUE OF STALLED REPLICATION FORKS

R. Torres[1], *C. Gándara*[1], *E. Serrano*[1], *J.C. Alonso*[1]
[1]*Centro Nacional de Biotecnología CNB-CSIC, Biotecnología Microbiana, Madrid, Spain*

Backgrounds

Impediments to replication fork progression cause fork stalling. In *Bacillus subtilis* cells, the DisA protein coordinates the response to DNA replication stress and DNA repair by the poorly characterized DNA damage tolerance (DDT) pathways. DisA synthetizes c-di-AMP, a conserved essential second messenger. High or low levels of c-di-AMP delay cell proliferation until damage is circumvented. DisA and RadA are implicated in the processing or stabilization/destabilization of recombination intermediates.

Objectives

To gain insights into the role of DisA and RadA proteins during DDT in *B. subtilis.*

Methods

Survival assays were performed to test whether *disA* and *radA* in combination with *ruvAB*, *recG* or *recU* deletions render cells sensitive to methylmethane sulfonate or 4-nitroquinoline, agents that stall replication forks. The nucleoid phenotype of these mutants and the localization of fluorescent variants of DisA and RadA in different genetic backgrounds were analysed by fluorescence microscopy. Expression of the RecA protein in these mutants was analyzed by Western Blot. The c-di-AMP synthesis and ATP hydrolysis were analysed by thin-layer chromatography using purified DisA and RadA proteins.

Conclusions

The *disA* or *radA* deletion does not affect the expression of the RecA protein.
DisA and RadA genetically interact with RecG and RecU and in minor extent with RuvAB, proteins involved in the recovery of stalled replication forks.
DisA-YFP and RadA-CFP formed dynamic foci that become static in the absence of RecU or RecG.
DisA interaction with RadA or with a reversed fork (HJ) inhibits c-di-AMP synthesis. dsDNA interaction with DisA makes it insensitive to RadA-mediated inhibition of c-di-AMP synthesis.

FEMS7-2734
Physiology / Biochemistry / Molecular Microbiology - Part III

AMPLIFICATION AND CLONING OF AN ALPHA-AMYLASE GENE FROM ANOXYBACILLUS FLAVITHERMUS K103 ISOLATED FROM AN ARMENIAN GEOTHERMAL SPRING

P. Hovhannisyan[1,2], H. Panosyan[1], A. Trchounian[1], N.K. Birkeland[2]
[1]Yerevan State University, Department of Biochemistry- Microbiology and Biotechnology, Yerevan, Armenia
[2]University of Bergen, Department of Biology and Centre for Geobiology, Bergen, Norway

Backgrounds

Thermozymes produced by thermophilic microorganism are stable and active at high temperatures. This feature makes them more advantageous compared to their mesophilic analogs for the usage in biotechnological processes. Amylases produced by thermophilic bacilli are the most widely used thermostable enzymes in starch industry and are in high demand

Objectives

The purpose of this work was to clone the alpha-amylase gene of the *Anoxybacillus flavithermus* K103 strain isolated from an Armenian geothermal spring.

Methods

Genomic DNA was isolated using GenElute Bacterial Genomic DNA Kit and sequenced using a HiSeq 400 Illumina genome sequencer. The DNA sequences coding for amylolytic enzymes were obtained and primers for an alpha-amylase were designed. The alpha-amylase gene was amplified and amplicons were ligated into a pET-21b(+) vector (Novagen) and transformed into chemically competent TOP10 *E. coli* cells.

Conclusions

Inserts were sequenced with designed primers, which confirmed that the gene sequence was correct and in the right reading frame and could be expressed in mesophilic *E.coli*. The alpha-amylase gene from *A. flavithermus* K103 shares 97% identity with the *Bacillus* sp. Alpha-amylases patented from different bacilli species are mostly comparable, suggesting that only limited natural variations in alpha-amylases may be discovered. Although the difference is not big at sequence level, it may have possible functional differences. Thus, it is important to express and purify the alpha-amylase from *A. flavithermus* K103 strain for further investigation.

The work was supported by the CPEA-2011/10081,CPEA-LT-2016/10095, ANSEF Microbio-3362 (HP), Thematic project of SCS RA 15T-1F399 (HP).

FEMS7-1072
Physiology / Biochemistry / Molecular Microbiology - Part III

COMPENSATORY H2 PRODUCING ACTIVITY OF ESCHERICHIA COLI HYDROGENASES DURING MIXED CARBON SOURCES FERMENTATION

K. Trchounian[1], S. Mirzoyan[1], A. Vassilian[2], A. Trchounian[1]
[1]Yerevan State University, Biochemistry- Microbiology and Biotechnology, Yerevan, Armenia
[2]Yerevan State University, Ecology and Nature Protection, Yerevan, Armenia

Backgrounds

During mixed-acid fermentation *Escherichia coli* evolves H_2. H_2 is produced via reversible membrane bound [Ni-Fe]-hydrogenases (Hyd).

Objectives

H_2 generation was investigated when the cells were grown during the fermentation of mixture of glucose, glycerol and formate at pH 7.5.

Methods

H_2 production was measured by using pair of Ti-Si and Pt redox electrodes.

Conclusions

In glycerol supplemented assays wild type cells produced H_2 ~2.8 and ~2.3 fold less, compared to the assays supplemented with formate or glucose, respectively. Using single or double Hyd deficient mutants it was shown that H_2 production was the same, as in wild type. Only deletion of large subunits of Hyd-1 to 3 decreased H_2 production by ~5 fold. When glucose was supplemented the deletion of large subunits of Hyd-3 or Hyd-4 resulted in enhanced H_2 production by ~1.5 fold; moreover, each other Hyd enzyme worked towards H_2 oxidation. But the deletion of three large subunits of Hyd-1 to 3 decreased H_2 production by 40%. This might suggest that the rest 60% of H_2 came from Hyd-4. When formate was supplemented H_2 production was decreased by ~1.6 fold in Hyd-3 or Hyd-4 mutants, compared to wild type. In Hyd-1 and Hyd-2 double mutant H_2 production was enhanced by ~4.7 fold indicating that at these conditions they worked in H_2 oxidizing mode.

Taken together it might be concluded that different Hyd enzymes compensate each other for maintaining H_2 cycling and thus proton motive force generation. Moreover, new properties of Hyd enzymes especially Hyd-4 were identified at these conditions.

FEMS7-0711
Physiology / Biochemistry / Molecular Microbiology - Part III

REGULATION OF ETHANOL DEGRADATION BY THE TEO-COMPONENT SYSTEM ABERDSR IN ACINETOBACTER BAUMANNII

P.Y. Tsai[1], K.S. Hsieh[1], H.Y. Shu[2], G.H. Lin[1]
[1]Master Program in Microbiology and Immunology, School of Medicine-Tzu Chi University, Hualien, Taiwan R.O.C.
[2]Bioscience Technology, Bioscience Technology-Chang Jung Christian University, Tainan, Taiwan R.O.C.

Backgrounds

Acinetobacter baumannii is an aerobic, rod-shape, no flagellum, gram negative opportunistic pathogen, and hospital disinfection agent, ethanol, can be used as its single carbon source. Biofilm and virulence factors were increased by low dose ethanol in *A. baumannii*.

Objectives

Low dose of ethanol may be an important growth factor in environment to *A. baumannii* for causing hospital-acquired infection. Environmental-sensing regulatory system which reported largely is two-component system (TCS). There are 16 TCSs in *A. baumannii*, but none was reported about regulation of ethanol metabolism.

Methods

Ethanol metabolism genes were screened from EZ-Tn5™<KAN-2> mutant library and confirmed by mark-less mutants. Physiological characteristics of mutants were cultured in M9 with 1% ethanol as single carbon source. Expressions of genes were detected by qRT-PCR and GFP reporter assay. Interaction of TCS, AbErdSR (*A. baumannii* ethanol regulated sensor/regulator), was used a TCS with reporter gene system (TCSG).

Conclusions

AberdSR single and double mutants loss ability of growth in M9 with 1% ethanol. Expression of *Abadh4* (*A. baumannii* alcohol dehydrogenase 4), *AberdS*, *AberdR*, and *actP* (acetate permease) were activated by ethanol in wild type, but did not shown in *AberdS* and *AberdR* single mutants. These results showed that AbErdSR is responsible for ethanol metabolism through the direct or indirectly regulation of alcohol dehydrogenase (ADH4) and *actP* for ethanol oxidation and acetate transport.

FEMS7-2203
Physiology / Biochemistry / Molecular Microbiology - Part III

BIOSYNTHESIS OF THE TWO YJJM (LGOR) ISOFORMS IS SUBJECTED TO A COMPLEX PHASE-DEPENDENT REGULATION

M. Tutukina[1,2], I. Suvorova[3], U. Shvyreva[1], D. Gagarinskaya[1], J. Cole[4], F. Kondrashov[2], O. Ozoline[1]
[1]Institute of Cell Biophysics RAS, Functional genomics and cellular stress, Pushchino, Russia
[2]Centre for Genomic Regulation CRG and Universitat Pompeu Fabra UPF, Bioinformatics and genomics programme, Barcelona, Spain
[3]Institute for Information Transmission Problems RAS, ESC "Bioinformatics", Moscow, Russia
[4]University of Birmingham, School of Biosciences, Birmingham, United Kingdom

Backgrounds

YjjM transcription factor was predicted to be involved in control of L-galactonate metabolism thus contributing to alternative pathways important for bacterial colonization and motility. Based on structural similarity, YjjM belongs to the GntR family of transcription regulators. We discovered that *yjjM* has at least two ORFs resulting in synthesis of two proteins variable in their DNA-binding domain.

Objectives

The aim of this study was to unravel how synthesis of the two YjjM isoforms is regulated.

Methods

Promoters were mapped with PlatProm software, single-round, RNA-seq, and 5'-RACE. Mutants were obtained by site-directed mutagenesis and "gene doctoring". Transcription was measured by reporter assays and qRT-PCR. Proteins specifically bound to the *yjjM* promoters were detected using DNA sampling and LC/MS-spectrometry. YjjM products were identified by Western-blot.

Conclusions

YjjM is coded by a foreign gene that appeared in the *E. coli* MG1655 genome as a result of horizontal transfer, and has at least two protein isoforms, ~35 and ~25 kDa. Their translation is initiated from different mRNAs: transcription of the full-size mRNA requires upstream binding of the cAMP-CRP-alphaCTD complex and is inhibited by H-NS. During exponential growth, H-NS also strongly inhibits synthesis of a shorter isoform that performs a self-regulatory function. On stationary phase, H-NS repression is relieved, possibly due to self-regulation and other proteins such as Dps functioning upon transition to stat-phase when the cell switches to colonization. ChIP- and RNA-seq data suggested tight involvement of YjjM in control of cell colonization that, thus, could be also fine-tuned by H-NS, Dps and cAMP-CRP.

FEMS7-1202
Physiology / Biochemistry / Molecular Microbiology - Part III

DISCOVERY AND CHARACTERIZATION OF THE NOVEL BACTERIAL ISOCYTOSINE DEAMINASES

J. Urbonavičius[1,2], *A. Aučynaitė*[2], *R. Rutkienė*[2], *D. Tauraitė*[2], *R. Meškys*[2]
[1]*Vilnius Gediminas Technical University, Department of Chemistry and Bioengineering, Vilnius, Lithuania*
[2]*Vilnius University, Department of Molecular Microbiology and Biotechnology- Life Sciences Center, Vilnius, Lithuania*

Backgrounds

Cytosine is one of four letters of a standard genetic code being part of both DNA and RNA. As part of the pyrimidine metabolism this heterocyclic base is converted into uracil by a hydrolase named cytosine deaminase. Isocytosine (2-aminouracil) is an isomer of cytosine.

Objectives

The aim of this study was to look for an enzyme converting isocytosine into uracil.

Methods

We used a uracil auxotroph-based selection strategy to search the metagenomic libraries for the genes encoding such enzymes. Three genes encoding potential bacterial deaminases were obtained. These genes were cloned into the expression vectors, expressed in *E. coli*, the recombinant proteins were purified, their enzymatic activities and substrate specificities were tested.

Conclusions

We found that isocytosine, but not cytosine is a substrate of the newly discovered isocytosine deaminases.

FEMS7-1476
Physiology / Biochemistry / Molecular Microbiology - Part III

IDENTIFICATION OF GENES ENCODING FOR POLYKETIDE SYNTHASES AND NON-RIBOSOMAL PEPTIDE SYNTHETASES FROM THE ANTARCTIC MARINE FUNGUS PSEUDOGYMNOASCUS VERRUCOSUM

I. Vaca[1], *F. Vega*[1], *G. Vasquez*[1], *M. Henriquez*[1], *C. Gil-Duran*[2], *R. Chavez*[2]
[1]*Universidad de Chile, Departamento de Quimica, Santiago, Chile*
[2]*Universidad de Santiago de Chile, Departamento de Biologia, Santiago, Chile*

Backgrounds

The ability to produce secondary metabolites by filamentous fungi from extreme environments has been poorly studied. Recent evidences indicate that fungi inhabiting these environments are producers of novel secondary metabolites. However, in these fungi the specific function of many genes of secondary metabolism remains as unknown. In our laboratory, we isolated an Antarctic marine fungus belonging to the class Leotiomycetes whose extracts from fermentation broth, analyzed by 1H-RMN, showed an interesting chemical profile, suggesting the presence of novel secondary metabolites.

Objectives

In the present work, we perform the phylogenetic identification of this Antarctic fungus. In addition, we show the presence of genes encoding for polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs) in its genome, and we describe the expression of these genes.

Methods

For the identification of the fungus, the phylogenetic position was determined by using four suitable molecular markers. On the other hand, partial sequences of five genes (four encoding for PKSs and one for NRPS) were isolated by PCR and analyzed by antiSMASH. Finally, the expression of these genes at different culture conditions was assessed by RT-PCR.

Conclusions

The results indicate that our isolate is closely related to the species *Pseudogymnoascus verrucosum*, whose presence had not yet been described in Antarctica. Analysis of the five sequences identified predicts that they would be part of clusters with unknown specific function. Only the PKS genes showed expression in the culture conditions tested, whereas the NRPS gene was silent. This work was supported by Fondecyt 1150894 and INACH RG_15-14.

FEMS7-2380
Physiology / Biochemistry / Molecular Microbiology - Part III

IDENTIFICATION AND CHARACTERIZATION OF THE TRANSCRIPTIONAL REGULATOR RESPONSIBLE FOR REGULATION OF THE ESTABLISHMENT GENES PRESENT ON CONJUGATIVE PLASMID P576 OF BACILLUS PUMILUS

J. Val Calvo[1], *W. Meijer*[1]
[1]*Centro de Biología Molecular Severo Ochoa UAM-CSIC, Virology and Microbiology, MADRID, Spain*

Backgrounds

Aspects related with the DNA processing and transfer of conjugative elements from a donor to a recipient cell have been studied well. However, recipient cells contain systems to avoid the entry of foreign DNA. Successful transfer of a conjugative element requires therefore mechanisms to inactivate these defense mechanisms, and, -importantly-, these defense mechanisms must be inactivated only transiently during and shortly after transfer of the conjugative element to avoid that the cell becomes vulnerable to the entry of other foreign DNAs, for instance of phages. Conjugative plasmids contain genes, named establishment genes, which fulfill a role in establishment of the plasmid in the new host. Little is known how these genes are regulated.

Objectives

Unraveling the transcriptional regulation of establishment genes present on Bacillus pumilus conjugative plasmid p576.

Methods

In vivo and in vitro techniques were used to identify promoters located upstream of p576 establishment genes. Transcriptional fusions of these promoters were used to set up a system to identify the gene encoding the regulator of these promoters. EMSAs in combination with site directed mutants were used to demonstrate binding of the purified regulatory protein to these promoters.

Conclusions

We have identified the mechanism that is responsible for transient expression of establishment genes of plasmid p576 after being transferred into a recipient cell. Besides description of this transcriptional regulator, a new gene regulatory network for establishment genes is described for a Gram+ positive plasmid.

FEMS7-2728
Physiology / Biochemistry / Molecular Microbiology - Part III

PROTEOME AND GENOMIC ANALYSIS OF CANDIDA ALBICANS EXOSOMES

V. Perez-donate[1], F. Perez-gimenez[2], L. Del Castillo-agudo[3], E. Valentin-gomez[3]
[1]HOSPITAL UNIVERSITARIO DE LA RIBERA, SERVICIO DE MICROBIOLOGÍA, ALZIRA, Spain
[2]Faculty of Pharmacy-University of Valencia, CHEMISTRY PHYSICAL, Burjassot Valencia, Spain
[3]Faculty of Pharmacy-University of Valencia, MICROBIOLOGY, Burjassot Valencia, Spain

Backgrounds

The morbility and mortality by *Candida albicans* has increased ostensibly in the last decades. In healthy individuals, *C. albicans* is a commensal but it can produce superficial injuries and systemic infections which can be harmless for the life of the patient. The knowledge of the molecular mechanisms of infections by *C. albicans* presents a great interest. The exosomes or extracellular vesicles (EV) are small membranous structures (40-200 nm) containing different kinds of macromolecules. However, the functional aspects of *C. albicans* EV remain unknown. Some authors attribute them a role in the communication between cells. These EV has been studied in various fungal pathogens as well as the model yeast *S. cerevisiae*.

Objectives

We have previously developed a protocol for EV purification from *Candida* species and analyzed its morphology and biochemical characteristics; in this work, we present a proteomic and a micro RNAs (miRNA) analysis.

Methods

EV were isolated as developed by our research group previously; analysis of proteins were carried out by MALDI-TOF, and miRNA identification by sequenciation; both analysis were carried out by the Servicio Central de Apoyo a la Investigación Experimental (SCSIE) at the University of Valencia (Burjassot, Valencia, Spain)

Conclusions

Proteome analysis showed the presence of 700 proteins, including cell wall proteins and sugar metabolism; however, a soft treatment of EV to detect surface proteins only showed 9 proteins mainly related to energy metabolism. The miRNA analysis identified 34 sequences most of them located in not transcribed regions but located in putative genetic regulation areas.

FEMS7-2230
Physiology / Biochemistry / Molecular Microbiology - Part III

ENTEROCOCCAL ESP PROTEIN SELF-ASSEMBLE INTO AMYLOID AGGREGATES TO BUILD BIOFILMS

J. Valle[1], *I. Lasa*[1]
[1]*Centro de Investigación Biomédica Navarrabiomed/ UPNA, Patogénesis Microbiana, Pamplona, Spain*

Backgrounds

Recent insights into bacterial biofilms have induced a paradigm shift towards the recognition of functional amyloids as common building block structures of the biofilms. Amyloids are attractive extracellular building material because they generally resist to harsh denaturing conditions and proteases and their polymerization occurs in the absence of energy. Our group has demonstrated that the staphylococcal Bap protein, a member of the BAP family of proteins, is processed and fragments containing the N-terminus of the protein become aggregation-prone and self-assemble into amyloid-like structures.

Objectives

Based on our results obtained with the staphylococcal Bap protein, we propose to study the amyloidogenic properties of other members of the BAP proteins. Specifically, we have analyzed the capacity of the enterococcal Esp protein to self-assembly into amyloid fibers.

Methods

The N-terminal region of Esp (Esp_B domain) shares 33% homology with the N-terminal domain of Bap. Expression of a chimeric protein containing the Esp_B domain showed that this region is sufficient to confer multicellular behavior. Purified recombinant N-terminal region of Esp formed aggregates with amyloid like conformation at acidic pH and bind to amyloid diagnostic dyes. We used the curli-dependent amyloid generator C-DAG-system to test the amyloid-forming propensity of Esp. The presence of extracellular amyloid aggregates of Esp_B was confirmed by analyzing the capacity of the strains to bind Congo Red dye and by detecting the presence of fibrillar structures by electron microscopy.

Conclusions

These results suggested that the mechanism of amyloid-like aggregation showed for the BAP-like proteins might be a widespread mechanism to build the biofilm matrix.

FEMS7-0463
Physiology / Biochemistry / Molecular Microbiology - Part III

SCH9P KINASE AND THE GCN4P TRANSCRIPTION FACTOR REGULATE GLYCEROL PRODUCTION DURING WINEMAKING

B. Vallejo[1], O. Helena[1], P. Cecilia[1], M. Emilia[1], A. Agustín[2]
[1]Universitat de Valencia, biochemistry and molecular biology, Valencia- Burjassot, Spain
[2]Instituto de Agroquímica y Tecnología de Alimentos IATA, Biotecnología, Valencia, Spain

Backgrounds

A basic understanding of the cellular mechanisms that allow cells to prevail in grape juice is essential to know how *Saccharomyces cerevisiae* adapts its metabolism in response to stressful winemaking conditions, mainly characterized by low nitrogen content. This feature is controlled by many nutrient-sensing pathways. The Tor/Sch9 pathway promotes growth and protein synthesis when nutrients are plenty and General Amino Acid Control, specifically transcription factor Gcn4p, is required for the activation of amino acid biosynthetic pathways.

Objectives

SCH9 impact on longevity depends on nitrogen/carbon ratio, as our previous results showed. When nitrogen is scarce, *SCH9* deletion shortens chronological life span under winemaking conditions and leads to increased glycerol accumulation, so we have investigated the molecular basis of this phenotype.

Methods

Transcriptomic and metabolomic approaches were carry out under winemaking conditions. *SCH9* deletion causes up-regulation of many amino acid biosynthetic pathways and *GCN4* overexpression produces short chronological longevity and increased glycerol. Therefore, both pathways are connected in terms of glycerol production and cause similar impact on yeast performance during winemaking. *SCH9* deletion down regulates peroxisomal genes, although localization of glycerol-3-P dehydrogenase Gpd1p, the limiting enzyme in glycerol biosynthesis, remains unchanged.

Conclusions

Despite both modificiations have different impact on the metabolome, *SCH9* deletion and *GCN4* overexpression cause down-regulation of glycolysis, and the common lower amount of the protective disaccharide trehalose may contribute to shorter longevity. *GCN4* overexpression increases the amount of most proteinogenic amino acids, while *SCH9* deletion down regulates most of them. *sch9*D increases the amount of lipids such phospholipids, ergosterol and sphingolipids.

FEMS7-3185
Physiology / Biochemistry / Molecular Microbiology - Part III

MICROSCOPE: AN INTEGRATED PLATFORM FOR THE EXPLORATION AND CURATION OF MICROBIAL GENOMES

D. Vallenet[1], A. Calteau[1], S. Cruveiller[1], M. Gachet[1], G. Gautreau[1], A. Josso[1], A. Lajus[1], J. Langlois[1], J. Mercier[1], H. Pereira[1], R. Planel[1], J. Rollin[1], D. Roche[1], Z. Rouy[1], C. Médigue[1]
[1]CEA/Genoscope - Université d'Evry Val-d'Essonne - CNRS-UMR 8030 - Université Paris-Saclay, LABGeM, Evry, France

Backgrounds

The analysis of genomes from NGS platforms needs to be automated and fully integrated. However, maintaining consistency and accuracy in annotation is a challenging task because millions of proteins in databanks are not assigned reliable functions.

Objectives

The LABGeM team of the Genoscope sequencing center focuses its research activities on the development and application of new methods for genome analysis. These tools are then made available through MicroScope (http://www.genoscope.cns.fr/agc/microscope), an integrated platform dedicated to microbial genome annotation and comparative analysis, which is being developed in our group since 2004.

Methods

The resource provides data from complete and ongoing genome projects together with post-genomic experiments (i.e. transcriptomics, re-sequencing of evolved strains) allowing users to improve the understanding of gene functions. We will present an overview of the MicroScope analysis pipelines and illustrate the use of several new functionalities in the context of data discovery and expert annotation, which concern:

- comparative genomics with synteny computations and pan-genome analyses,
- the prediction of virulence and antimicrobial resistance genes,
- the detection and annotation of genomic regions of interest, like, secretion systems, integrons and secondary metabolite biosynthesis gene clusters,
- and metabolic network reconstruction assisted by the GROOLS expert system (https://github.com/grools).

Conclusions

To date, MicroScope contains data for about 7,000 microbial genomes, part of which are manually curated and maintained by microbiologists (>3,200 personal accounts in March 2017). The platform enables collaborative work in a rich comparative genomic context and improves community-based curation efforts.

FEMS7-0938
Physiology / Biochemistry / Molecular Microbiology - Part III

RESPIRATORY COMPLEX I VARIANTS, SELECTED DURING EVOLUTION UNDER FREQUENT ANTIBIOTIC TREATMENT INDUCE BACTERIA PERSISTER CELL FORMATION THROUGH CYTOPLASMID ACIDIFICATION

B. Van den Bergh[1], J.E. Michiels[1], J. Schimpf[2], S. Bruschel[2], E. Vervisch[1], T. Friedrich[2], M. Fauvart[3], J. Michiels[1]
[1]KU Leuven, Microbial and Molecular Systems, Leuven, Belgium
[2]Freiburg University, Biochemistry, Freiburg, Germany
[3]imec, miLab team, Leuven, Belgium

Backgrounds

With rising antibiotic resistance and lacking new antibiotics targeting pristine targets, soon, mankind slips into an era where common infections will once again kill millions. Apart from resistance, bacteria can also resort to other means to evade antibiotics. Previously we showed evolution of extreme antibiotic tolerance in bacteria under frequent antibiotic exposure, not by antibiotic resistance but by acquiring mutations that increase the numbers of antibiotic-tolerant persister cells. One target is complex I, a membrane-embedded super enzyme of 535kDa and main entry point for electrons from NADH into the respiratory chain.

Objectives

We wanted to verify the importance and diversity of complex I variants in conferring extreme persistence levels and decipher their molecular mechanisms.

Methods

Whole genome sequencing on *Escherichia coli* evolved in the lab (both lab and pathogenic strains), identified a multitude of mutations in *nuo*. Surprisingly, all hit *nuoLMN* and none of the other 10 *nuo* genes. Genome engineering and antibiotic tolerance assays demonstrated their causality and ruled out overall knock-out effects. Biochemical analyses on purified complex I variants further confirmed stability and validity as entry point of electrons in the respiratory chain but instead indicated a diminished proton-translocating activity. Flow cytometry and pH-sensitive fluorophores pinpointed the effect specifically towards the pH component as membrane potentials remained unchanged, also explaining the unaffected antibiotic uptake of the mutants. Artificially changing cytoplasmic pH showed the causality of cytoplasmic acidification in the increased antibiotic tolerance.

Conclusions

Complex I variants, selected during frequent antibiotic exposure, influence bacterial persister cell formation through cytoplasmic acidification.

FEMS7-0964
Physiology / Biochemistry / Molecular Microbiology - Part III

GROWING AKKERMANSIA MUCINIPHILA FOR THERAPEUTIC APPLICATIONS

K. van der Ark[1], S. Aalvink[1], C. Belzer[1], W. de Vos[1,2]
[1]Wageningen University and Research, Microbiology, Wageningen, The Netherlands
[2]University of Helsinki, RPU Immunobiology- Department of Bacteriology & Immunology, Helsinki, Finland

Backgrounds

The mucus degrading gut symbiont *Akkermansia muciniphila* is commonly associated with a healthy gut. Its relative abundance was found to be negatively correlated with metabolic disorders, obesity and chronic gut inflammations. We have previously shown that this anaerobic bacterium is capable to reduce mucosal levels of oxygen.

Objectives

To enable further physiological research, we aimed to develop a defined minimal medium to grow *A. muciniphila*, which is free of animal-derived compounds to allow application in clinical trials.

Methods

To support the design of a minimal medium we used predictions based on the genome-scale metabolic model (GEM). This suggested that *A. muciniphila* has a unique gap in the peptidoglycan synthesis pathway, since there is no gene annotated to code for GlmS, which mediates the conversion from fructose-6-phosphate (Fru6P) to glucosamine-6-phosphate (Glu6P) and is essential for peptidoglycan synthesis. We confirmed the absence of this conversion in *A. muciniphila* by overproducing the only alternative enzyme that could mediate this conversion, *A. muciniphila* NagB.

Conclusions

This enzyme was unable to efficiently convert Fru6P to Glu6P under physiological conditions. As a result, N-acetylglucosamine needs to be supplemented to the medium for *A. muciniphila* to synthesize peptidoglycan and grow efficiently. This and other knowledge obtained from the GEM was integrated to design and validate an animal component-free medium. The medium was used in preclinical trials in mice and to allow large scale production of *A. muciniphila* for human clinical trials. This study illustrates the use of GEMs to optimize growth and shows its applications for next generation therapeutic microbes.

FEMS7-0244
Physiology / Biochemistry / Molecular Microbiology - Part III

ZINC-INDUCED TRANSPOSITION OF INSERTION SEQUENCE ELEMENTS CONTRIBUTES TO INCREASED ADAPTABILITY OF CUPRIAVIDUS METALLIDURANS

J. Vandecraen[1], P. Monsieurs[1], M. Mergeay[1], N. Leys[1], A. Aertsen[2], R. Van Houdt[1]
[1]SCK•CEN, Microbiology Unit, Mol, Belgium
[2]Katholieke Universiteit Leuven, Laboratory of Food Microbiology, Leuven, Belgium

Backgrounds

Mobile genetic elements play a significant role in bacterial evolution and Insertion Sequence (IS) elements are of specific interest as they constitute an importing driving force for genome plasticity. *Cupriavidus metallidurans* type strain CH34 is a well-studied metal-resistant β-proteobacterium and contains a very high number of genes involved in the resistance and processing of heavy metals as well as a high number of intact IS elements. It counteracts metal ion toxicity via a battery of resistance mechanisms, with an important role for its two megaplasmids pMOL28 and pMOL30. Interestingly, strain AE126, a derivative of CH34 cured of its megaplasmid pMOL30 harboring the main zinc resistance determinant, is still able to increase its zinc resistance.

Objectives

To determine the contribution of IS elements to the genetic adaptation of *Cupriavidus metallidurans* AE126 to toxic zinc concentrations.

Methods

Zinc resistant AE126 derivatives, which arose upon plating on medium supplemented with a toxic zinc concentration, were characterized in detail.

Conclusions

All resistant variants carried a compromised *cnrYX* regulatory locus, which resulted in derepression of CnrH sigma factor activity and concomitant induction of the corresponding RND-driven *cnrCBA* efflux system. Late-occurring zinc resistant variants likely arose in response to the selective conditions, as they were enriched in *cnrYX* disruptions caused by specific IS elements whose transposase expression was found to be zinc-responsive. Deletion of *cnrH*, and consequently the CnrH-dependent adaptation potential, still enabled adaptation by transposition of IS elements that provided outward-directed promoters driving *cnrCBAT* transcription. Thus, transposition of IS elements can be induced by stress conditions and play a multifaceted, pivotal role in the adaptation to zinc.

FEMS7-1727
Physiology / Biochemistry / Molecular Microbiology - Part III

REGULATION OF CITRATE UTILISATION IN LACTOCOCCUS LACTIS

O. van Mastrigt[1], E. Mager[1], C. Jamin[1], T. Abee[1], S. Lillevang[2], E. Smid[1]
[1]Wageningen University & Research, Laboratory of Food Microbiology, Wageningen, The Netherlands
[2]Arla Foods, Arla Strategic Innovation Centre, Brabrand, Denmark

Backgrounds

Citrate is an important precursor for flavour formation in dairy fermentations. *Lactococcus lactis* subsp. *lactis* bv *diacetylactis* is one of the main species in dairy starter cultures that can utilise citrate. In this bacterium citrate utilisation is limited by transport of citrate via the plasmid-encoded citrate permease CitP.

Objectives

The aim of this study was to determine the mechanisms governing citrate utilisation in *L. lactis* to be able to select conditions which improve flavour formation.

Methods

L. lactis has been grown in chemostats at different pH values (5.5 and 7), types of nutrient limitation (lactose and amino acid) and in the presence and absence of citrate. Regulation of citrate utilisation was studied the level of (i) the plasmid copy number, (ii) *citP* transcription and (iii) citrate utilisation capacity.

Conclusions

We demonstrated that the plasmid copy number slightly increased in cells grown at low pH or under amino acid limitation. However, the main regulation mechanism for citrate utilisation is increased expression at low pH when citrate is present. The requirement of citrate has been overlooked in previous studies due to the presence of citrate in M17 growth medium. The increased transcription of *citP* correlated with a higher citrate utilisation capacity. Finally, acidic environments not only resulted in higher citrate utilisation rates, also acetoin formation significantly increased via induction of the acetoin pathway.

FEMS7-1166
Physiology / Biochemistry / Molecular Microbiology - Part III

MOLECULAR MECHANISM OF ACTION OF BACTERIAL GTP(GDP) PYROPHOSPHOKINASES

K. Van Nerom[1], *A. Garcia-Pino*[1]
[1]*Free University of Brussels ULB, BSBP, Gosselies, Belgium*

Backgrounds

Most bacteria can produce phenotypic variants of actively dividing cells, known as persisters, which are tolerant to most known antibiotics. Moreover, the pace of antibiotic discovery to combat resistant pathogens has slowed down. Discovering new targets, developing species-specific antibiotics and identifying prodrugs that have the potential to eradicate dormant persisters remains a challenge for modern molecular biology.

Objectives

The formation of persister cells is controlled by the bacterial signalling molecule ppGpp, which is synthesized by the almost ubiquitous enzyme RelA. Although this enzyme has been discovered several decades ago, the mechanism that leads to ppGpp synthesis and its regulation remain unknown. With this project, we aim to close that gap and study the regulation of RelA and the molecular mechanism of ppGpp-synthesis leading to persistence.

Methods

RelA from *Chlorobium tepidum* was incubated with six different Nanobodies prior to screening the ppGpp-producing activity of RelA by means of TLC. Nanobodies are antibody fragments from Camelid single chain antibodies that can bind specific epitopes of a target protein with high affinity. Nanobodies can act as inhibitors or activators of the target protein and can be used as a molecular tool to characterize their target.

Conclusions

We discovered two Nanobodies that activate ppGpp production by RelA from *Chlorobium tepidum*, while one Nanobody acts as an inhibitor of the enzyme. This research can lead to finding new ways to inhibit RelA *in vivo* and can provide a rational pathway to design new antibiotics with the potential to eradicate persistent bacterial infections.

FEMS7-2697
Physiology / Biochemistry / Molecular Microbiology - Part III

SIRTUIN-DEPENDENT REVERSIBLE LYSINE ACETYLATION OF A TOXIN-ANTITOXIN SYSTEM MODULATES THE ACTIVITIES OF THIS SYSTEM, WHICH IS INVOLVED IN SALMONELLA PERSISTENCE.

C.M. VanDrisse[1], *A.R. Parks*[1], *J.C. Escalante-Semerena*[1]
[1]*UGA, Microbiology, Athens, USA*

Backgrounds

Bacterial toxin-antitoxin systems have been implicated in persistence of pathogens. Typically, toxin activity is neutralized through physical contact with its cognate antitoxin. Recently, others showed that the TacT toxin of *Salmonella enterica* acetylates aminoacyl-tRNAs leading to arrest of protein synthesis and persistence. We have further characterized the TacA (antitoxin) TacT (toxin) system of this bacterium, and show that TacT also acetylates TacA, a modification that is removed by the *Salmonella* CobB sirtuin deacetylase.

Objectives

To characterize the role of reversible lysine acetylation in *Salmonella* persistence.

Methods

Transfer of acetyl moieties by TacT (toxin) onto TacA (antitoxin) was detected by phosphor imaging. Removal of the label required incubation with the NAD^+-dependent CobB sirtuin deacetylase. The acetylation site in TacA was established by LC/MS/MS peptide fingerprinting. *In vitro* protein synthesis was used to quantify the effect of antitoxin acetylation on TacT aminoacyl-tRNA acetylating activity. Electrophoretic mobility shift assays were utilized to characterize DNA binding of wild-type and TacA acetylation-mimic variants.

Conclusions

While remaining in complex, TacT acetylated TacA, which caused an increase in TacT-dependent translation inhibition. Additionally, acetylated TacA altered TacAT binding to the *tacAT* promoter, which is currently being analyzed by RT-qPCR. *In vivo* analyses showed operons encoding TacAT variant complexes mimicking acetylated TacA had negative effects on growth, validating *in vitro* results. This is the first report of an acetyltransferase targeting a protein and non-protein substrate and the first report of reversible lysine acetylation of an antitoxin modulating the activity of the toxin, rather than physical separation of the toxin-antitoxin proteins.

FEMS7-2314
Physiology / Biochemistry / Molecular Microbiology - Part III

THE SMALL PROTEIN TRPM MODULATES MORPHO-PHYSIOLOGICAL DIFFERENTIATION IN THE MODEL ACTINOMYCETE STREPTOMYCES COELICOLOR A3(2)

A. Vassallo[1], E. Palazzotto[1], M.V. Pravatà[1], L. Botta[2], G. Gallo[1], A.M. Puglia[1]
[1]University of Palermo, STEBICEF, Palermo, Italy
[2]University of Palermo, DICAM, Palermo, Italy

Backgrounds

TrpM, a small protein of 63 amino acids, modulates tryptophan (Trp) metabolism and morpho-physiological differentiation in the filamentous bacterium *Streptomyces coelicolor* A3(2), a model organism for antibiotic production and cell differentiation. Indeed, the *trpM* knock-out mutant strain is characterized by a delayed growth on minimal medium, smaller aerial hyphae, and reduction of both spore and antibiotic actinorhodin production in comparison with the wild-type strain. These observations were in agreement with proteomic analyses which highlighted a role for TrpM in controlling i) Trp production through Trp precursor availability and, thus ii) bacterial growth and morpho-physiological differentiation.

Objectives

Construction and morpho-physiological characterization of a *S. coelicolor* A3(2) *trpM* knock-in mutant.

Methods

- Construction of a *trpM* knock-in mutant by *E. coli-S. coelicolor* interspecific conjugation using the pIJ8600/trpM integrative plasmid.

- Scanning Electron Microscope (SEM) analysis.

- Spectrophotometric analysis and microbiological assays for evaluating antibiotic production.

Conclusions

A *trpM* knock-in mutant strain of *S. celicolor* A3(2) was constructed and showed an increased production of actinorhodin and spores: moreover, SEM analysis revealed an earlier formation of septa in aerial hyphae and confirmed that TrpM has a role in controlling the morpho-physiological differentiation of *S. coelicolor* A3(2).

FEMS7-1134
Physiology / Biochemistry / Molecular Microbiology - Part III

MULTIPLE CROSSTALK BETWEEN TOR AND THE CELL INTEGRITY MAPK SIGNALING PATHWAY IN FISSION YEAST

B. Vázquez Marín[1], M. Madrid[1], A. Franco[1], T. Soto[1], E. Gómez-Gil[1], J. Vicente-Soler[1], M. Gacto[1], J. Cansado[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain

Backgrounds

In eukaryotic cells, the highly conserved Target of Rapamycin (TOR) and the Mitogen Activated Protein Kinase (MAPK) signaling pathways elicit adaptive responses to extra- and intracellular conditions by regulating essential cellular functions. In the fission yeast Schizosaccharomyces pombe the cell integrity MAPK pathway (CIP) regulates morphogenesis, cell wall structure and ionic homeostasis.

Objectives

To underscore the nature of the functional relationships between both TOR and CIP pathways in fission yeast.

Methods

We show that the Rab GTPase Ryh1, a TORC2 complex activator, cross-activates the CIP and its core member, the MAPK Pmk1, by two distinct mechanisms. The first one involves TORC2 and its downstream effector, Akt ortholog Gad8, which together with TORC1 target Psk1 increase protein levels of the PKC ortholog Pck2 during cell wall stress or glucose starvation. Also, Ryh1 activates Pmk1 in a TORC2-independent fashion by prompting plasma membrane trafficking and stabilization of upstream activators of the MAPK cascade, including PDK ortholog Ksg1 or Rho1 GEF Rgf1. Besides, stress-activated Pmk1 cross-inhibits Ryh1 signaling by decreasing the GTPase activation cycle, and this ensures cell growth during alterations in phosphoinositide metabolism.

Conclusions

Our results reveal a highly intricate cross-regulatory relationship between both pathways that warrants adequate cell adaptation and survival in response to environmental changes.

FEMS7-1172
Physiology / Biochemistry / Molecular Microbiology - Part III

DISTINCT FUNCTIONAL RELEVANCE OF DYNAMIC GTPASE CYSTEINE METHYLATION IN FISSION YEAST

B. Vázquez Marín[1], T. Soto[1], R. Martín-García[2], P. Pérez[2], M. Madrid[1], E. Gómez-Gil[1], J. Vicente-Soler[1], M. Gacto[1], J. Cansado[1], A. Franco[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain
[2]Instituto de Biología Funcional y Genómica IBFG- Universidad de Salamanca, Morphogenesis and cell polarity, Salamanca, Spain

Backgrounds

The final step in post-traslational processing of Ras and Rho GTPases involves methylation of the prenylated cysteine by a isoprenylcysteine-O-carboxyl methyltransferase (ICMT). ICMT activity is essential for cell growth and development in higher eukaryotes, and inhibition of GTPase methylation has become an attractive target in cancer therapy to inactivate prenylated oncoproteins. However, the specificity and dynamics of the GTPase methylation process remain to be fully clarified. Notably, cells lacking Mam4, the ICMT ortholog in the fission yeast *Schizosaccharomyces pombe,* are viable.

Objectives

To analyze the role of methylation on GTPase localization and function in a simple eukaryotic model organism.

Methods

We show that methylation differentially affects GTPase membrane localization and signaling to MAPK cascades, being particularly relevant for plasma membrane tethering and downstream signaling of palmitoylated and farnesylated Ras and Rho GTPases lacking C-terminal polybasic motifs. Mam4 also negatively regulates TORC2 signaling by a cross-inhibitory mechanism relying on proper Rho GTPase methylation. Moreover, dynamic increase in cysteine methylation impairs cell growth, reduces Rho palmitoylation, and modulates its function *in vivo* either positively or negatively depending on the absence or presence of palmitoylation.

Conclusions

These results highlight the requirement for a strict control of GTPase methylation threshold *in vivo* to allow adequate GTPase function.

FEMS7-2581
Physiology / Biochemistry / Molecular Microbiology - Part III

DIVERSITY OF MOBILE GENETIC ELEMENTS HARBOURED ON CHROMOSOME OF AVIBACTERIUM PARAGALLINARUM

C. Vazquez-Cruz[1], M.P.G. Sanchez-Alonso[1], Z. Guerrero-Gonzalez[1], N.E. Rojas-Ruiz[1], N. Negrete-Abascal[2], S. Vaca-Pacheco[2], V. Perez-Marquez[3]
[1]Instituto de Ciencias,
Centro de Investigaciones en Ciencias Microbiologicas- Benemerita Universidad Autonoma de Puebl a, Puebla, Mexico
[2]Universidad Nacional Autonoma de Mexico, Facultad de Estudios Superiores-Iztacala- Tlalnepantla, Estado de Mexico, Mexico
[3]Biotecnologia Veterinaria SA de CV, BIOVETSA, Tehuacan- Puebla, Mexico

Backgrounds

Four different *Avibacterium paragallinarum* genome sequences (AVP72, 221, JF4211 and CL) have been reported with a high number of contigs, except AVPG2015, that was grouped in 24 contigs. Those results could be due to: procedure to obtain the nucleotide sequence, the quality of sequences obtained, the procedure to assembly the information and the richness of repeated sequences in each genome.

Objectives

The aim of this work was to compare transposase, integrase and prophage sequences to understand the dynamics of them in the five reported *A. paragallinarum* genomes including one more recently sequenced by our laboratory (Avpg FES5 arranged in 46 contigs).

Methods

In silico analysis of three prophages was made in six genomes: CL, 221, JF4211, AVP72, AVPG2015 and FES5. Transposases and integrases analysis was made in CL and AVPG2015 genomes.

Conclusions

Lambdoid prophage sequences were found in all genomes. AvpmuC-2M and HP2-like sequences were found in CL, 221 and JF4211. CL genome is rich in transposase and integrase sequences (80 and 62, respectively) while AVPG2015 contains only 13 and 14 of each one; all of them grouped in five superfamilies of each group. Transposases and integrases sequences present in AVPG2015 genome just were classified into two families for each one. FES5 genome sequence is similar to AVPG2015 sequence and the low number of contigs among both, suggest that both strains are restrictive to mobile elements. A high number of movable elements into a genome sequence could be the main restriction to get a one molecule assembles in *A. paragallinarum* genomes.

FEMS7-1421
Physiology / Biochemistry / Molecular Microbiology - Part III

ROLE OF AMINO ACID BIOSYNTHESIS IN SUPPRESSING THE THERMOSENSITIVE CELL DIVISION MUTANT ZIPA1 OF ESCHERICHIA COLI

D. Vega[1], W. Margolin[1]
[1]The University of Texas Health Science Center at Houston, Microbiology and Molecular Genetics, Houston, USA

Backgrounds

As reported by Pichof and Lutkenhaus in 2002, we observed that thermosensitive *zipA1* mutant was slightly elongated in LB at 30˚C and filamented at 42ºC. However, we also discovered that in minimal medium (either M9 or MOPS), *zipA1* cells grow poorly at 30ºC and form filaments and have other shape abnormalities. We confirmed that this cell division defect in minimal medium was exclusive to *zipA1*. Adding back casamino acids to M9 completely corrected the defects. Glycine or L-threonine greatly corrected the growth and division defect of *zipA1* in M9. Other amino acids can also correct the defects, but to a lesser degree.

Genes associated with L-threonine biosynthesis (*thrA*, *thrB* and *thrC*) were deleted in a *zipA1* background. Deletion of *thrB* or *thrC* but not *thrA,* partially suppressed thermosensitivity and cell defects. We considered L-homoserine (an intermediate in the biosynthesis of L-methionine, L-threonine and L-isoleucine) as the key player. Added L-homoserine did not suppress *zipA1* thermosensitivity in LB, but in M9 it partially suppressed division defects in *zipA1* despite inducing variations in cell width and length in *zipA*+ cells. L-homoserine is converted into S-adenosylmethionine (SAM), and the lack of SAM is known to inhibit cell division in *E. coli*. We confirmed that SAM was not involved in the *zipA1* phenotype.

The thermosensitivity of *zipA1*mutants lacking *glyA*, *ltaE* and *tdcB* was partially suppressed in LB, similar to the effect of *thrB* or *thrC* deletions. Therefore, blocking different branches of the threonine/glycine biosynthetic pathway can lead to partial suppression of the *zipA1* cell division defect.

Objectives

Characterization of the thermosensitive cell division mutant *zipA1* of *Escherichia coli*

Methods

Genetic Recombination-Transduction

Conclusions

Our data support a role of the L-threonine or glycine biosynthetic pathway in the function of ZipA and/or early stages of *E. coli* cell division, thus implicating amino acid homeostasis as a regulator of cytokinesis.

FEMS7-1373
Physiology / Biochemistry / Molecular Microbiology - Part III

UNVEILING THE EVOLUTIONARY NEW ROLE OF CBIKP COBALTOCHELATASE FROM DESULFOVIBRIO VULGARIS

M. Videira[1], S. Lobo[1], I. Pacheco[1], M. Wass[2], M. Warren[3], M. Teixeira[1], P. Matias[1,4], C. Romão[1], L. Saraiva[1]
[1]Instituto de Tecnologia Química e Biológica António Xavier, Biological Chemistry, Oeiras, Portugal
[2]School of Biosciences- University of Kent, Biomolecular Medicine, Canterbury, United Kingdom
[3]School of Biosciences- University of Kent, Protein Science, Canterbury, United Kingdom
[4]Instituto de Biologia Experimental e Tecnológica, Structural Biology for Drug Discovery, Oeiras, Portugal

Backgrounds

Desulfovibrio vulgaris (*D. vulgaris*) are sulphate-reducing bacteria with numerous porphyrin containing enzymes. These organisms use sirohydrochlorin as substrate for metal insertion to synthesize sirohaem, cobalamin and haem *b*. Two homologs of CbiK sirohydrochlorin chelatase were previously identified in the genome of *Desulfovibrio vulgaris*, namely CbiK^{c} and Cbik^{p}, cytoplasmic and periplasmic forms, respectively. However, only Cbik^{p} is able to bind haem.

Objectives

This work aimed to study the structure/function relationship and the localization of *D. vulgaris* Cbik^{p} cobaltochelatase.

Methods

Site-directed mutagenesis was used to generate 11 specific Cbik^{p} mutants which were analysed regarding the chelatase activity and the oligomerization and haem binding properties. Furthermore, GFP fusions were made to Cbik^{p} to determine cellular localization.

Conclusions

We show that Cbik^{p} is a periplasmic protein. Two residues were identified as essential for the Cbik^{p} Co^{2+}/Fe^{2+} chelatase activity, namely His154 and His216. We also revealed that residues Arg54 and Glu76 are important for the oligomerization of Cbik^{p} as a tetramer. His96 was identified as the residue responsible for the binding of two haem molecules within the tetramer. We proved that two additional haem groups can be bound to the protein involving residue His103. Altogether, the absence of these two histidine residues from other bacterial CbiK cobaltochelatases, the periplasmic localization and the ability to bind more haem groups indicate that *D. vulgaris* Cbik^{p} may function as a haem transporter.

FEMS7-1518
Physiology / Biochemistry / Molecular Microbiology - Part III

USTILAGO MAYDIS VACUOLAR ASPARTIC PROTEASE A, HOMOLOGUE OF THE HUMAN CATHEPSIN D PROTEIN IS INVOLVED IN DIMORPHISM AND PATHOGENESIS OF THE FUNGUS

L. Villa-Tanaca[1], V. Soberanes-Gutiérrez[1], P. Tesillo-Moreno[1], M. Juárez-Montiel[1], A. Ibarra-García[1], C. Hernández-Rodríguez[1], J. Ruiz-Herrera[2]
[1]Instituto Politecnico Nacional, Microbiología- ENCB, México- CDMX, Mexico
[2]Instituto Politecnico Nacional, Ingeniería Genética, Irapuato, Mexico

Backgrounds

Vacuole proteases have important functions in different physiological processes and during the autophagy phenomenon in fungi. As continuation of our studies of the proteolytic system of the *Ustilago maydis* a phytopathogenic Basidiomycota, we have analysed the role of the *pep4* gene encoding the vacuolar acid proteinase PrA in the pathogenesis and morphogenesis of the fungus.

Objectives

To analyze the role of the *pep4* gene encoding the vacuolar acid proteinase PrA in the pathogenesis and morphogenesis of the fungus and obtaining the recombinant *U. maydis* protease A by heterologous expression.

Methods

The location of the protease in the vacuole was performed by the use of fluorescent probes, we obtained deletion mutants of the gene in sexually compatible strains of *U. maydis* (FB1 and FB2). The *pep4um* gene was cloned and expressed in *Pichia pastoris*.

Conclusions

The yeast-to-mycelium dimorphic transition, was severely reduced in the Δ*pep4* mutants. Additionally, virulence of the mutants in maize seedlings was reduced, as revealed by the lower proportion of plants infected, and the reduction in these of the size in the tumours. A 54 kDa recombinant protein was observed and was confirmed to be an aspartic protease. It is important to note that the *in silico* analysis suggests that Pep4um is homologue of the human Cathepsin D protein, thus Pep4-um-rec protein may be used to test inhibitors of human Cathepsin D, an important breast cancer therapeutic target. These results are evidence of the importance of the *pep4* gene for the morphogenesis and virulence of *U. maydis*.
SIP-20171553

FEMS7-0662
Physiology / Biochemistry / Molecular Microbiology - Part III

SIGNALING MEDIATED REGULATION OF METABOLISM IN BACILLUS ANTHRACIS

R. Virmani[1], Y. Hasija[2], Y. Singh[1]
[1]Prof. Y. Singh, Department of Zoology, Delhi, India
[2]Delhi Technological University, Biotechnology Department, Delhi, India

Backgrounds

Intracellular pathogens need to coordinate their metabolism with cell division process to survive and replicate inside the host cell. Thus, cell cycle machinery works to fine-tune the division process via multiple signaling pathways that critically monitor the environment.

Objectives

To understand the signaling mediated metabolic alteration of pathogenic bacteria, we aim to characterize the role of one of the key glycolytic enzyme, Enolase, which plays an important role in cellular events of *Bacillus anthracis*, the causative agent of Anthrax.

Methods

We studied the regulation of the enzyme by the signaling machinery of bacteria. Over expression strategies have been employed to check the effect of this glycolytic enzyme on spore revival process. Further, mass spectrometry was utilized to confirm the post-translational modifications.

Conclusions

Increase in the levels of Enolase, leads to disruption in the spore revival process; additionally, interaction of the enzyme with some key proteins involved in the cell cycle machinery was also confirmed. Our analysis revealed a novel mechanism by which cell division is linked to glycolysis. This study not only highlights the regulation of Enolase by the signal transduction cascade but also indicates a novel molecular circuit that controls cell growth and division. We propose, that the integration of metabolic and signaling cues allows the cells to modulate metabolism and assist in cell survival.

FEMS7-2215
Physiology / Biochemistry / Molecular Microbiology - Part III

HOST FACTORS INVOLVED IN INTEGRON INTEGRASE-MEDIATED RECOMBINATION.

C. Vit[1], *C. Loot*[1], *G. Garriss*[1], *J. Matysiak*[2], *V. Parissi*[2], *D. Mazel*[1]
[1]*Institut Pasteur, Genomes and Genetic, paris, France*
[2]*Université de Bordeaux, Microbiologie fondamentale et pathogénicité, Bordeaux, France*

Backgrounds

Integrons are bacterial genetic elements that allow the capture of circular non-replicative gene cassettes and the expression of the ORF they carry. Integrons contain Y-recombinase, IntI, that recognizes and recombines a double-stranded *attI* site with a folded single-stranded *attC* site. This reaction is responsible for the formation of an atypical Holliday junction and its resolution has been shown to require a replication step unlike the classical pathway (Loot *et al.* 2012). Moreover, the integrase IntI1 (associated with Mobile Integrons) and IntI4 integrase (associated with the Sedentary Integron of *Vibrio cholerae*) have been shown to recombine with different efficiencies depending on the host (Biskri *et al.* 2005). These observations might reflect different requirements for host-encoded proteins, either for the recombination reaction itself, or for the resolution of the recombination intermediate.

Objectives

Here we aim at identifying host factors encoded in *V. cholerae* genome that may contribute to *attI x attC* recombination.

Methods

To identify these host factors we are using: 1) a biochemical approach in order to select proteins interacting with IntI1 or IntI4 (TAP-Tag and an *in vitro* purification of integrase partners (V. Parissi)) 2) a genetic screen to find out genes coding for proteins that are essential during *attC* x *attI* recombination with IntI4.

Conclusions

First results indicate that some proteins of the replication (DnaN or PriA) could interact with the IntI integrases, tests will be done to confirm these interactions.

FEMS7-1553
Physiology / Biochemistry / Molecular Microbiology - Part III

DYNAMICS OF FUNCTIONAL MEMBRANE MICRODOMAINS IN BACTERIA

R. Wagner[1,2], *D. Lopez*[1,2]
[1]*Centro Nacional de Biotecnología CNB-CSIC, Microbial Biotechnology, Madrid, Spain*
[2]*Research Center for Infectious Diseases ZINF, Institute for Molecular Infection Biology IMIB, Würzburg, Germany*

Backgrounds

The cellular membrane fulfills an important function in protection and is the resource of communication with the surrounding. Especially for single cell organisms the integrity and functionality of membranes is crucial for the functionality of almost all cellular processes. The correct organization of membranes, including those from prokaryotes, relies on a heterogeneous distribution of constituent lipids and embedded proteins, which leads to the formation of membrane microdomains harboring proteins specialized in specific cellular processes. Functional membrane microdomains (FMMs) have been described to organize in the membrane of bacterial cells and their integrity has been shown to be important in cellular processes like signal transduction and membrane trafficking.

Objectives

Interestingly, FMMs are highly dynamic assemblies moving within the bacterial membrane. However, the biological significance and the underlying molecular mechanism that orchestrate FMM dynamics remain largely unknown.

Methods

Here, we use cutting-edge live-cell imaging techniques in combination with a number of biochemical and molecular approaches to investigate FMM dynamics in the bacterial models *Bacillus subtilis* and *Staphylococcus aureus*. Genetic and physiological targeting of cellular integrity led to unambiguous results showing that FMM dynamics is intimately associated with membrane integrity as well as the integrity of other membrane-related cellular structures.

Conclusions

Thus, alterations in membrane organization leads to severe interference in the FMM dynamics which in turn affects the functionality of FMM-associated cellular processes. Finding the biological significance of highly dynamic FMMs in the bacterial cell guides the design of antimicrobial agents for new yet unidentified target mechanisms.

FEMS7-0375
Physiology / Biochemistry / Molecular Microbiology - Part III

FUNCTIONAL ANALYSIS OF TWO COMPONENT SYSTEM ABERASR IN ACINETOBACTER BAUMANNII

M.Y. Wen[1], *G.H. Lin*[1], *Y.H. Chiu*[1]
[1]*Tzu Chi University, Department of Microbiology, Hualien, Taiwan R.O.C.*

Backgrounds

Acinetobacter baumannii is a Gram-negative bacterium. In previous, *A. baumannii* could grow in low concentration of ethanol. Currently, no article published about two component system can regulate alcohol metabolism in *A. baumannii*. *In silico* analysis, AbEraSR in *Acinetobacter baumannii* share 26% and 33% amino acid sequence identity with EraSR in *P. aeruginosa*, respectively. EraSR is known as a two component system that can regulate alcohol metabolism.

Objectives

Whether AbEraSR plays a role in alcohol metabolism and finds out which gene will be regulated by AbEraSR.

Methods

Ethanol degradation of *A. baumannii* maker-less mutagenesis mutants were cultured in M9 medium with different concentration of ethanol as single carbon source. Regulation cascade of AbEraSR were confirmed by red fluorescence protein reporter gene system(TCSR) in *E. coli*. Electrophoretic mobility shift assay(EMSA) was demonstrated to determine the regulation of alcohol dehydrogenase which is a key enzyme in alcohol metabolism by AbEraR directly.

Conclusions

In single carbon source culture, the result showed that there were no significantly different between wild-type and Δ*AberaS* strain. However, this result indicated that there are another systems such as two component system or regulator protein can regulate AbEraSR or alcohol metabolism directly. In order to comprehensive understanding how AbEraSR regulates the downstream gene, we construct TCSR in *E. coli* and coordinate lab member construction of green fluorescence protein reporter gene system to comprehend regulation of these two component systems. If AbEraSR will regulate ADH the detector can detect red fluorescence.

FEMS7-0074
Physiology / Biochemistry / Molecular Microbiology - Part III

ACUH FROM RUEGERIA LACUSCAERULENSIS IS A CROTONASE SUPERFAMILY ENZYME WITH ACTIVITY TOWARDS METHYLTHIOACRYLOYL-COA AND ACRYLOYL-COA

H. Bullock[1], Y. Zhu[1], C. Reisch[1], W.B. Whitman[1]
[1]University of Georgia, Microbiology, Athens, USA

Backgrounds

Dimethylsulfoniopropionate (DMSP) produced by marine phytoplankton is an important source of reduced carbon and sulfur for marine bacteria, and the DMSP demethylation pathway is a major mechanism for its assimilation. The final reaction in this pathway is catalyzed by DmdD, a crotonase superfamily enzyme that converts methylthioacryloyl-CoA (MTA-CoA) to acetaldehyde, CO_2, and methanethiol (MeSH). *Ruegeria lacuscaerulensis* metabolizes DMSP, however an ortholog to *dmdD* is missing from its genome.

Objectives

Does a paralog of DmdD provide the physiological activity for the demethylation pathway in roseobacteria lacking DmdD?

Methods

Results: RL_AcuH was isolated from *R. lacuscaerulensis* cell extracts based upon its activity toward MTA-CoA. Recombinant RL_AcuH possessed activity with MTA-CoA and crotonyl-CoA as well as high activity with acryloyl-CoA. For this reason, it was named acryloyl-CoA hydratase or AcuH. Due to its activity with acryloyl-CoA, AcuH was predicted to function in both the demethylation and cleavage pathways. Subsequent tests revealed that RL_AcuH required activation by ADP or NAD^+ for high catalytic efficiency towards MTA-CoA. RL_*acuH* was able to complement growth and MeSH production defects of a *Ruegeria pomeroyi dmdD* deletion mutation. While AcuH orthologs from *Dinoroseobacter shibae* DFL12, *Oceanibulbus indolifex* HEL45, *Pseudomonas putida* KT2440, and *Burkholderia thailandensis* E264 all had substantial activity towards crotonyl-CoA, their catalytic efficiency for MTA-CoA hydration was low, indicating that this is not likely to be a physiological function.

Conclusions

While AcuH is likely a physiological MTA-CoA hydratase in *R. lacuscaerulensis*, this activity is not uniform across all bacteria possessing AcuH-like enzymes.

FEMS7-0902
Physiology / Biochemistry / Molecular Microbiology - Part III

DIMERIZATION OF THE POREFORMING TOXIN HOKB IS REQUIRED TO INDUCE PERSISTENCE IN ESCHERICHIA COLI

D. Wilmaerts[1], N. Verstraeten[1], M. Bayoumi[2], G. Maglia[3], J. Michiels[1]
[1]KU Leuven, Centre of Microbial and Plant Genetics, Heverlee, Belgium
[2]KU Leuven, Biochemistry- Molecular and Structural Biology Section, Heverlee, Belgium
[3]University of Groningen, Faculty of Mathematics and Natural Sciences, Groningen, The Netherlands

Backgrounds

When an isogenic bacterial population is treated with high doses of bactericidal antibiotics, a small fraction survives. These so-called persister cells are not genetic mutants but phenotypic variants. Toxin-antitoxin modules have been implicated in the induction of persistence. These systems inhibit essential cellular functions, leading to persistence.

Objectives

We have previously shown that the essential GTPase ObgE induces persistence in *E. coli* by upregulating the toxin HokB. After insertion in the membrane, HokB induces persistence by collapsing the membrane potential. We aim at further elucidating the mode of action of HokB.

Methods

We used conductance measurements on planar lipid bilayers to test *in vitro* pore formation by HokB. As we observed formation of covalently bound HokB dimers on a western blot, we tested whether disulfide bridges play a role in the persistence phenotype of HokB. For this, we used cysteine substitution mutants of HokB and knockout strains of periplasmic oxidoreductases.

Conclusions

Using a planar lipid bilayer, HokB was shown to form pores of 0.4-1.6 nm. In addition, we discovered that the formation of disulfide bridges in the periplasmic part of the peptide is mediated by periplasmic oxidoreductases and is essential for the persistence phenotype of HokB. Interestingly, the persister fraction was directly affected by chemically changing the ratio between monomers and dimers using dithiothreitol (DTT). Combined, our findings demonstrate the essential role of dimerization of HokB for persistence, offering the possibility to control persistence by changing the redox conditions.

FEMS7-1049
Physiology / Biochemistry / Molecular Microbiology - Part III

DISTRIBUTION OF MAZEF/PEMIK LOCI AMONG STAPHYLOCOCCI AND THEIR GENETIC CONTEXTS

B. Wladyka[1], M. Bukowski[1], K. Hyz[1], M. Janczak[1], M. Hydzik[1]
[1]Jagiellonian University, Department of Analytical Biochemistry, Krakow, Poland

Backgrounds

Toxin-antitoxin (TA) systems are small genetic entities widespread among bacteria. Recently the importance of their role in various aspects of bacterial physiology has been growingly recognized. Generally, the systems are made of two components, with the antitoxin gene preceding the one for the toxin. The open reading frames are often overlapping and code for relatively small products. This, together with the knowledge of already described TA systems, allows for application of bioinformatics in search for undiscovered ones. Staphylococci are gram-positive bacteria associated with human and animals. Among them *S. aureus* is considered as a dangerous opportunistic pathogen with increasing drug-resistance.

Objectives

Recently, a significant amount of genetic data has been increasingly released due to the rapidly growing use of massive parallel sequencing methods. Nonetheless, the last comprehensive analysis of TA system distribution, which included 18 staphylococcal genomes, was undertaken seven years ago. Here we provide a distribution analysis of *mazEF/pemIK* loci among staphylococci based on over 6,000 genome sequences.

Methods

Five computational approaches based on BLAST+ tools were applied. Protein sequences of the well-characterized staphylococcal TA systems, MazEF-Sa and PemIK-Sa1, were used as prototypical ones.

Conclusions

Five computational approaches gave the same set of twelve *mazEF/pemIK* loci, with ten newly uncovered. Chromosomally encoded *mazEF-Sa* is present in virtually every staphylococcal genome. Despite greater diversity, the occurrence frequency of *pemIK* loci is generally low. Interestingly, the loci is often located on mobile genetic elements carrying drug resistance genes. Moreover, preference of *pemIK-Sa1*-carrying strains towards poultry *S. aureus* strains is noticeable.

FEMS7-1246
Physiology / Biochemistry / Molecular Microbiology - Part III

PROTEIN-PROTEIN INTERACTION IN TYPE II-A CRISPR-CAS SYSTEM

S.P. Wong[1,2], F. Hille[1], H. Richter[1], K. Schmidt[1], C. Emmanuelle[1,2,3]
[1]Max Planck Institute For Infection Biology, Department of Regulation in Infection Biology, Berlin, Germany
[2]The Laboratory for Molecular Infection Sweden MIMS- Umeå Centre for Microbial Research UCMR-Umeå University, Department of Molecular Biology, Umeå, Sweden
[3]Humboldt University, Humboldt University, Berlin, Germany

Backgrounds

The RNA guided prokaryotic adaptive immune system CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) immunizes microorganisms against mobile genetic elements. The hallmark of the system is the genomic CRISPR array, which consists of repeat sequences interspaced with invader-derived spacer sequences. The immunity operates in three stages, *i.e.* (1) acquisition and integration of a short nucleic acid sequence from the invader into the CRISPR array (adaptation); (2) transcription and maturation of the transcripts into mature CRISPR RNAs (crRNAs) that contain a repeat and a spacer portion (biogenesis); (3) neutralization of the invaders by a crRNA-guided Cas nuclease that targets foreign nucleic acids through sequence complementarity between the cognate spacer of the crRNA and the invader's sequence (protospacer). Cas1, Cas2, Csn2 and Cas9 of the *Streptococcus thermophilus* type II-A CRISPR-Cas system are essential for adaptation, however, their interactions within a complex are still obscure.

Objectives

Analysis of the detailed Cas protein-protein interactions

Methods

First, *in vivo* acquisition studies verified the activity of the acquisition machinery (2 CRISPR-Cas systems, CRISPR1 and CRISPR3) of *S. thermophilus* LMD-9 after phage infection. We observed higher acquisition rates in the CRISPR3 locus compared to CRISPR1. An *in vitro* pull-down assay using recombinant Cas1 as bait protein demonstrated the Cas1-Cas9 protein-protein interaction. For further identification of the interacting residues, a SPOT membrane with an immobilized array of overlapping Cas1 peptides was used. The result demonstrated that the C-terminus of Cas1 interacts with Cas9.

Conclusions

In summary, we show the interaction between Cas1 and Cas9 and disclose involved residues.

FEMS7-0019
Physiology / Biochemistry / Molecular Microbiology - Part III

OFF-PATHWAY ASSEMBLY OF FIMBRIA SUBUNITS IS PREVENTED BY CHAPERONE CFAA OF CFA/I FIMBRIAE FROM ENTEROTOXIGENIC E. COLI

D. Xia[1]
[1]*National Cancer Institute- NIH, Laboratory of Cell Biology, Bethesda, USA*

Backgrounds

The assembly of the class 5 CFA/I fimbriae of enterotoxigenic *E. coli* was proposed to proceed via the alternate chaperone-usher pathway.

Objectives

How different the alternate chaperone-usher pathway is from the classic chaperone-usher pathway is investigated.

Methods

Here, we show that in the absence of the chaperone CfaA, CfaB, the major pilin subunit of CFA/I fimbriae, is able to spontaneously refold and polymerize into cyclic trimers. CfaA kinetically traps CfaB to form a metastable complex that can be stabilized by mutations. Crystal structure of the stabilized complex reveals distinctive interactions provided by CfaA to trap CfaB in an assembly competent state through donor-strand complementation and cleft-mediated anchorage. Mutagenesis indicated that donor-strand complementation controls the stability of the chaperone-subunit complex and the cleft-mediated anchorage of the subunit C-terminus additionally assist in subunit refolding. Surprisingly, over-stabilization of the chaperone-subunit complex led to delayed fimbria assembly, whereas destabilizing the complex resulted in no fimbriation.

Conclusions

Thus, CfaA acts predominantly as a kinetic trap by stabilizing subunit to avoid its off-pathway self-polymerization that results in energetically favorable trimers and could serve as a driving force for CFA/I pilus assembly, representing an energetic landscape unique to class 5 fimbria assembly.

FEMS7-0115
Physiology / Biochemistry / Molecular Microbiology - Part III

A GENETIC TOOL TO STUDY THE PHENOTYPE OF FOREIGN GENES

K. Yadav[1], *D. Hughes*[1]
[1]*Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden*

Backgrounds

Horizontal gene transfer (HGT) is an important driver of evolution and is a major source of antibiotic resistance genes in bacterial pathogens. We developed a genetic tool using constitutive promoters of different strength to study the effect of expression level on the phenotypes of genes from distantly related bacteria expressed in *Escherichia coli*.

Objectives

We developed a genetic tool using constitutive promoters of different strength to study the potential of HGT to confer antibiotic-resistance in *E. coli* and how the level of resistance, and fitness, is affected by gene expression level.

Methods

Lambda-red recombineering was used to create a set of isogenic *E. coli* strains in which any HGT antibiotic resistance gene could be placed under the control of 8 different constitutive promoters. The relative strength of each promoter was quantified by measuring fluorescence intensity using a magnetic associated cell sorter (MACS).

Conclusions

The expression of sYFP in the set of constructed strains covered a wide range, from 2- to 500-fold above background level. We used the synthetic expression system to ask whether foreign ribosomal protection proteins (TetO, TetW, TetQ, TetB(P), OtrA) transferred by would be able to confer tetracycline resistance in *E. coli*. Using TetM as a positive control we showed that three of the foreign proteins, TetO, TetW and TetQ, conferred expression-dependent levels of resistance approximately equal to that conferred by TetM (>128 mg/mL at the highest level of expression). In contrast, TetB(P) conferred only a modest increase (8 mg/mL) and OtrA none at all even at the highest level of expression. Here we have developed and validated a genetic tool to study the influence of expression level on horizontally transferred foreign genes in *E. coli*. This system can be used to predict the potential of foreign genes to confer novel phenotypes such as antibiotic resistance on the recipient bacteria.

FEMS7-0771
Physiology / Biochemistry / Molecular Microbiology - Part III

CHARACTERIZATION OF UNCHARACTERIZED TRANSCRIPTION FACTORS, YAGI, YBIH, AND YDCN, OF ESCHERICHIA COLI BY USING THE GENOMIC SELEX SYSTEM.

K. Yamamoto[1], *A. Ishihama*[2]
[1]*Hosei University, Department of Frontier Bioscience and Micro-Nano Technology Research Center-, Koganei- Tokyo, Japan*
[2]*Hosei University, Department of Frontier Bioscience and Micro-Nano Technology Research Center, Koganei- Tokyo, Japan*

Backgrounds

Escherichia coli contains a total of more than 4,400 protein-coding sequences on its genome. The selection of genes for expression and their expression level are determined by controlling the distribution of a limited number of transcription apparatus on the genome.

Objectives

A total of 7 species of sigma factor, the promoter recognition subunit of RNA polymerase (RNAP), and about 300 species of transcription factor (TF) are altogether involved in this gene selection process. At present, however, the regulatory functions are not known for about one fifth of the *E. coli* TFs.

Methods

For identification of the regulation targets by uncharacterized TFs, we have then developed an improved system of Genomic SELEX (systematic evolution of ligands by exponential enrichment). The Genomic SELEX screening system is particularly useful for identification of regulation targets of hitherto uncharacterized TFs. Although the majority of TFs regulates a number of target promoters and genes, but we have identified about 10 species of TFs that regulate only a single (or a few) target. Here we will describe three single-target TFs, YagI, YbiH, and YdcN. The targets predicted based on the SELEX screening were experimentally examined *in vitro* and *in vivo*.

Conclusions

Taken all these results together, we propose that: YagI is a repressor for the *yagA* and *yagE* operon; YbiH (remaned to CecR) is a bifunctional regulator, repressing the *ybiH* operon and activating the *rhlE* operon, both being involved in the sensitivity control to cefoperazone and chloramphenicol; and YdcN (remaned to StuR) is a regulator of sulfur utilization.

References:
Ishihama, A. *et al*. (2016) *Nucleic Acid Res.* **44**(5):2058-2074
Yamanaka, Y. *et al.* (2016) *Microbiology* **162**(7):1253-1264.
Yamamoto et al. (2015) Microbiology 161(Pt 1):99-111

FEMS7-0429
Physiology / Biochemistry / Molecular Microbiology - Part III

AN EXTRA REPABC LOCUS IN THE INCRH2 TI PLASMID PTIBO542 EXERTS INCOMPATIBILITY TOWARD AN INCRH1 PLASMID

S. Yamamoto[1], V. Agustina[1], A. Sakai[1], K. Moriguchi[1], K. Suzuki[1]
[1]Hiroshima University, Department of Biological Science- Graduate School of Science, Higashi-Hiroshima, Japan

Backgrounds

Ti/Ri plasmids in pathogenic *Agrobacterium* species are *repABC* replicons that are stably maintained by the function of *repABC* genes. Two Ti plasmids, pTiBo542 and pTiS4, belonging to incRh2 and incRh4 incompatibility groups, respectively, were reported to carry two *repABC* loci. However, the significance of the extra *repABC* genes remains unclear.

Objectives

The objective of this study is to reveal the roles of the two *repABC* loci in the two Ti plasmids, pTiBo542 and pTiS4.

Methods

We constructed mini replicons carrying any one or both of the *repABC* loci (referred to as *repABC1* and *repABC2* here) and examined their replication and incompatibility properties. The replication of the mini replicons was checked by transformation of Agrobacterial cells. The incompatibility between plasmids was evaluated by evicting the resident plasmid from the recipient cell by introducing the incoming plasmid in conjugation assay.

Conclusions

The *repABC2* loci of the two Ti plasmids lack an ability to replicate in *Agrobacterium* cells. However, a *repABC2* locus of pTiBo542 exerts incompatibility toward an incRh1 plasmid unilaterally and can thus displace the coexisting incRh1 plasmid efficiently. We suggest that the locus contributes to plasmid retention by eliminating the burden of co-existing competitive plasmids in host cells through its incompatibility.

FEMS7-1464
Physiology / Biochemistry / Molecular Microbiology - Part III

CROSS-REGULATION BETWEEN TWO TYPE II TOXIN/ANTITOXIN SYSTEMS FROM DIFFERENT MOBILE GENETIC ELEMENTS IN SHEWANELLA ONEIDENSIS

J. Yao[1], *X. Wang*[1]
[1]*Chinese Academy of Sciences- South China Sea Institute of Oceanology, LMB, Guangzhou-, China*

Backgrounds

Toxin/antitoxin (TA) systems are prevalent in bacteria, archaea and bacteriophage. They can commonly found in mobile genetic elements (MGEs) such as plasmids, prophages and integrated conjugative elements. However, physiological functions of these TA systems and cross-regulation among these MGEs remain largely unexplored.

Objectives

Here we characterized a type II TA pair ParEso/CopGAso from cryptic prophage CP4So in *Shewanella oneidensis*.

Methods

We demonstrate that ParEso/CopGAso played an important role in the maintenance of CP4So circle after CP4So is excised from host genome. Toxin ParEso inhibited cell growth, resulting in filamentous growth and eventually cell death. The cognate antitoxin CopGAso neutralized the toxicity of ParEso through direct protein-protein interaction. Additionally, CopGAso autoregulated the TA operon through binding to the palindromic sequences (5'-GTATTACCTAGTAGTAC-3') in the promoter region. The N-terminal of CopGAso contains a typical Ribbon-Helix-Helix domain which exerts DNA-binding activity through binding to specific palindromic sequences. Additionally, antitoxin CopGAso also repressed the transcription of another type II TA system $PemIK_{SO}$ from the megaplasmid pMR-1 in *S. oneidensis* through binding to a similar palindrome in its promoter. We further demonstrated that CopGAso can also function as a positive regulator for the sigma factor RpoE2 and help mediate the oxidative stress response.

Conclusions

We thus provide evidences that type II TA systems from different mobile genetic elements can cross-talk through the DNA binding ability of the antitoxin component.

FEMS7-2639
Physiology / Biochemistry / Molecular Microbiology - Part III

REMOVAL OF ACETYLATION OF SIALIC ACID BY ESTERASES POTENTIATES PNEUMOCOCCAL NEURAMINIDASE ACTIVITY FOR MUCIN UTILIZATION, COLONIZATION AND VIRULENCE

H. Yesilkaya[1]
[1]*University of Leicester, Infection- Immunity & Inflammation, Leicester, United Kingdom*

Backgrounds

Pneumococcal neuraminidase is a key enzyme for deglycosylation of host glycans, and plays an important role in host survival, colonisation, and pathogenesis of infections caused by *Streptococcus pneumoniae*. One of the factors that can affect the activity of neuraminidase is the acetylation present in its substrate sialic acid.

Objectives

The objective was to gather data to support hypotheses that pneumococcal esterases potentiate neuraminidase activity by removing acetylation from sialic acid, and this deacetylation will have a major effect on pneumococcal survival on mucin, colonisation, and virulence.

Methods

These hypotheses were tested using isogenic mutants and recombinant esterases in microbiological, biochemical and *in vivo* assays.

Conclusions

Pneumococcal esterase activity is encoded by at least four genes, SPD_0534 (EstA) was found to be responsible for the main esterase activity, and the pneumococcal esterases are specific for short acyl chains. Both the Axe and EstA esterases could use acetylated xylan and Bovine Sub-maxillary Mucin (BSM), a highly acetylated substrate, but only EstA was active against tributyrin (triglyceride). Incubation of BSM with either Axe or EstA led to the acetate release in a time and concentration dependent manner, and pre-treatment of BSM with either enzyme increased sialic acid release on subsequent exposure to neuraminidase. Mutation of *estA* alone or in combination with *nanA* (codes for neuraminidase A), or the replacement of its putative serine active site to alanine, reduced the pneumococcal ability to utilise BSM as a sole carbon source, sialic acid release, colonisation, and virulence in a mouse model of pneumococcal pneumonia.

FEMS7-1526
Physiology / Biochemistry / Molecular Microbiology - Part III

COMPARISON OF PROTEOMIC EXPRESSION IN BRUCELLA ABORTUS MUTANTS WITH DIFFERENT CHARACTERISTICS BY TWO DIMENSIONAL ELECTCTROPHORESIS

P. Woo Bin[1], I. Young Bin[1], S. Soojin[1], H.S. Yoo[1]
[1]College of Veterinary Medicine - Seoul National Univer, Department of Infectious Diseases, Seoul, Republic of Korea

Backgrounds

Brucellosis caused by *Brucella abortus.* is debilitating and zoonotic disease. The bacterium is facultative intracellular organisms. Pathogenesis of the bacterial infection is very difficult. Therefore, underlying mechanism of the *B. abortus* infection is still remained to be resolved even though several researches have been done. As one of the steps, proteins showing different expression in the mutants showing different biological phenotypes were identified.

Objectives

Objective of this study was to identify the proteins showing the different expression level in *B. abrotus* mutants with different biological characteristics.

Methods

B. abortus mutants were generated by random insertion of transposon, EZ-Tn5^{TM}. Total proteins of 5 mutants and wild type of *B. abortus* were purified, analyzed by 2-DE and compared. Protein spots with a greater than 2-fold change were identified using LC-ESI-MS and Mascot.

Conclusions

Various spots showing increase or decrease in the expression were identified by 2-DE analysis of mutants and wild strains. Of the spots, commonly increased or decreased spots in the mutants were selected and identified. DnaK, ClpB, and Pgk were highly increased while SecA, EtfB, and RplK were highly decreased. In the proteins, heat shock proteins, ClpB and DnaK, may play an important role in the intracellular infection of *B. abortus* by protein aggregation suppression. Also, the changes in factor related to the growth, RplK, was related with phenotypic characteristics. These results may solve the clues related with virulence factors by observing the changes of various factors in the mutants. This work was supported by NRF (No. 2014R1A2A2A01007291) and BK21 PLUS.

FEMS7-0424
Physiology / Biochemistry / Molecular Microbiology - Part III

CHARACTERIZATION OF THE FRUBKA OPERON REGULATOR FRUR IN VIBRIO CHOLERAE

C.K. Yoon[1], Y.H. Park[1], Y.R. Kim[1], Y.J. Seok[1]
[1]Department of Biological Sciences and Institute of Microbiology, Biological Science, Seoul, Republic of Korea

Backgrounds

Fructose repressor (FruR), which belongs to the GalR/LacI family of transcriptional regulators, regulates the expression of genes involved in the transport and utilization of fructose through direct binding to the cognate DNA sequence upstream of the *fruBKA* operon. In *Escherichia coli*, FruR has been elucidated as a global regulator responsible for controlling the carbon metabolic flux through repression or activation of mRNA expression of approximately 60 genes in 24 operons.

Objectives

Vibrio cholerae also has a FruR ortholog (vcFruR) that shares 47% amino acid sequence identity with *E. coli* FruR (ecFruR). vcFruR has unique features compared to the ecFruR.

Methods

In this study, we investigated the transcriptional regulation of FruR on the expression of the *fruBKA* operon. We were able to confirm the direct binding of FruR to three putative binding sites through DNase I footprinting and gel shift assays.

Conclusions

First, the oligomeric state of ecFruR is a homo-tetramer whereas vcFruR exists in a dimeric state *in vitro*. In addition, the gene encoding vcFruR is located adjacent to and divergently transcribed from the *fruBKA* operon. Lastly, only 3 sites are expected to be the target sites of FruR in the entire *V. cholerae* genome and these sites are located in the intergenic region between *fruR* and *fruBKA*. These findings indicate that vcFruR might have regulatory mechanism different from that of ecFruR. By binding to each target site in different combinations, vcFruR is considered to regulate the transcriptional level of the *fruBKA* operon and itself in *V. cholerae.*

FEMS7-0249
Physiology / Biochemistry / Molecular Microbiology - Part III

FUNCTIONAL AND STRUCTURAL STUDIES OF THE NISIN RESISTANCE PROTEIN NSR OF STREPTOCOCCUS AGALACTIAE

J. Zaschke-Kriesche[1], B. Frieg[2], H. Gohlke[1], S.H.J. Smits[1]
[1]Heinrich-Heine-University- Duesseldorf, Institute of Biochemistry, Duesseldorf, Germany
[2]Heinrich-Heine-University- Duesseldorf, Institute of pharmaceutical and medical chemistry, Duesseldorf, Germany

Backgrounds

The antimicrobial peptide nisin contains 34 amino acids and is produced by some *Lactococcus lactis* strains. It is used in the food and dairy industry as a preservative since decades. It belongs to the lantibiotic subclass and displays high antimicrobial activity against mainly Gram-positive bacteria. The human pathogen *Streptococcus agalactiae*, however is resistant against nisin due to the presence of the *nsr*-operon (Khosa et. al 2013) encoding a two-component system NsrRK, an ABC-transporter NsrFP and the nisin resistance protein (NSR). Latter is a serine protease, which inactivates nisin by cleaving off the last six amino acids. We recently solved the X-ray structure of *Sa*NSR, which revealed that nisin is specifically recognized by NSR via the two last lanthionine-rings D and E (Khosa et. al 2016).

Objectives

The goal of this study is to overcome the lantibiotic-resistance in human pathogens using nisin as a model system. Thereby lantibiotics can be used as novel antibiotics and exploit there full potency.

Methods

We used molecular dynamics and simulations to screen over 3000 natural compounds to find inhibitors. The identified compounds were subjected to *in vivo* and *in vitro* assays to identify their ability to inhibit *Sa*NSR.

Conclusions

Here, we present the functional characterization of *Sa*NSR as well as the first subset of compounds, which can be used to bypass lantibiotic resistance in human pathogens allowing displaying the full potential of these peptides.

FEMS7-3102
Physiology / Biochemistry / Molecular Microbiology - Part III

COMPARATIVE WHOLE-GENOME ANALYSIS REVEALS ARTIFICIAL SELECTION EFFECTS ON USTILAGO ESCULENTA GENOME

Y. Zhang[1], Z. Ye[1], Y. Pan[2], H. Cui[1], G. Jin[3], A. McHardy[2], L. Fan[3], X. Yu[1]
[1]China Jiliang University,
Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, Hangzhou, China
[2]Heinrich Heine University, Department of Algorithmic Bioinformatics, Düsseldorf, Germany
[3]Zhejiang University, Zhejiang Key Laboratory of Crop Germplasm Resources, Hangzhou, China

Backgrounds

Ustilago esculenta, infects *Zizania latifolia*, and induced host stem swollen to be a popular vegetable called *Jiaobai* in China. It is the long-standing artificial selection that maximizes the occurrence of favorable *Jiaobai*, and thus maintaining the plant-fungi interaction and modulating the fungus evolving from plant pathogen to entophyte.

Objectives

The aim of this study was to elucidation of the *U. esculenta* genomic information as well as expression profiles, to give more comprehensive insights into the molecular mechanism underlying artificial selection, and into smut fungi-host interactions.

Methods

Whole genome of *U. esculenta* was sequenced and transcriptomes of the fungi and its host were analyzed.

Conclusions

The 20.2 Mb *U. esculenta* draft genome of 6,650 predicted genes including mating, primary metabolism, secreted proteins, shared a high similarity to related Smut fungi. But *U. esculenta* prefers RNA silencing not RIP in defense and has more introns per gene, indicating relatively slow evolution rate. The fungus also lacks some genes in amino acid biosynthesis pathway which were filled by up-regulated host genes, and developed distinct amino acid response mechanism to balance the infection-resistance interaction. Besides, *U. esculenta* lost some surface sensors, important virulence factors and host range related effectors to maintain the economic endophytic life.

FEMS7-1682
Physiology / Biochemistry / Molecular Microbiology - Part III

IDENTIFICATION OF THE TARGET OF THE TYPE II PARE2 TOXIN IN E. COLI

Z. Zhao[1]
[1]*Free University of Brussels ULB, Science, Gosselies, Belgium*

Backgrounds

TypeII Toxin-Antitoxin (TA) systems are protein pairs consisting of a stable toxin, and a labile antitoxin. In a former study, our group has identified a novel type II antitoxin family named *paaA*, which is associated with *parE* toxins. Together with a regulator named *paaR* in upstream, these ORFs constitute a three-component operon. Overexpression of ParE2 leads to cell death in a SOS-dependent manner. Using a ParE2-GFP fusion, it was shown that ParE2-GFP co-localized with the nucleoid, suggesting that ParE2-target complex is associated to DNA. The structure of ParE2 is similar to that of *C. crescentus* ParE but displays a distinct pattern of conserved surface residues, in agreement with its apparent inability to interact with GyrA

Objectives

The objective of this work is to identify the target of the ParE2 toxin.

Methods

The methods include (1) kill/rescue assay expressing subunits of DNA-gyrase (GyrA and GyrB) and of topoisomerase IV (ParC and ParE) with ParE2 toxin to test if there is compensation; (2) co-purification of ParE2-target complexes and (3) identification of target by mass spectrometry.

Conclusions

Preliminary data indicate that overexpression of subunits of topoisomerase IV ParC and ParE might lead to better protection against ParE2 activity than GyrA or GyrB overexpression. However, some refinements are needed since overexpression of these subunits also leads to some toxicity.

FEMS7-3162
Physiology / Biochemistry / Molecular Microbiology - Part III

EXPLORING BACTERIAL KILLING MECHANISMS ON THE APICAL SURFACE OF CULTURED PIG TRACHEAL EPITHELIA

S. Zhu[1]
[1]*the University of Hong Kong, School of Biomedical Sciences, Hong Kong, Hong Kong S.A.R.*

Backgrounds

Airway surface liquid on the apical side of epithelia contains antimicrobial substances for killing invaded bacteria.

Objectives

It is unclear how these antimicrobial substances are formed and regulated.

Methods

To address this question, we used the method of live and dead staining to measure killing of Gram-negative bacteria *Psedomonas (P.) aeruginosa*. Our data demonstrate that many *P. aeruginosa* either in a free form or attached to a gold EM grid were apparently killed after a short incubation period on the surface of cultured pig tracheal epithelia, and the bacterial killing rate was about 30% higher than that in PBS solutions as the control. Similar bacterial killing rates of *P. aeruginosa* were obtained when the bacteria were placed into washout fluid, collected from the apical side of epithelia using a small amount of the PBS solution. To explore possible candidates responsible for bacterial killing, we analyzed washout fluid and found the presence of several antimicrobial substances, such as lactoferrin, LPLUNC1 and lysozyme. Correlation analysis between the normalized protein level of each band in the silver-staining gel and corresponding bacterial killing rate by the same washout fluid indicates that the bacterial killing rate appeared to be positively correlated with the amount of protein at about 75 kD including lactoferrin, or at size slightly less than 10 kD possibly containing antimicrobial peptides.

Conclusions

These data suggest that cultured epithelia secrete several antimicrobial substances onto the apical surface of epithelia, in which lactoferrin and antimicrobial peptides might play an important role in enhancing bacterial killing in airway.

FEMS7-2195
Physiology / Biochemistry / Molecular Microbiology - Part III

A NEW FAMILY OF STAPHYLOCOCCUS AUREUS PHAGES ISOLATED FROM PATIENTS WITH CYSTIC FIBROSIS

M. Ziemyte[1], M. Cervera-Alamar[1], K. Guzmán[1], M.Á. Tormo-Mas[1]
[1]HOSPITAL LA FE, Infección Grave, VALENCIA, Spain

Backgrounds

Mobile genetic elements (MGE) such as pathogenicity islands and bacteriophages are very common in clinical isolates of *Staphylococcus aureus*. Moreover, they are closely related with each other and its interaction leads to dissemination of various virulence factors. For example, bacteriophages are responsible for induction, packaging and transference of *Staphylococcus aureus* pathogenicity islands (SaPIs).

Objectives

As pulmonary infection with *S aureus* is a frequent problem in patients with cystic fibrosis we were interested in the role of MGE and its interaction in these strains.

Methods

During our study, we have tested 200 *S. aureus* isolates from 118 cystic fibrosis patients. Interestingly, 77% of these strains in its genome contained bacteriophage with integrase type III. After sequencing of 19 strains, we observed that 17 of them had conserved packaging module that was different from previously described phages. In all of the cases phages contained TerL, endonuclease HNH and a hypothetical protein that might be a new TerS. We determined that deletion of this protein doesn't affect phage DNA replication but completely eliminated phage packaging and infectivity. As obvious morphological changes in phage structure were observed in this mutant, we want to demonstrate cos-site cleavage by this HNH-TerS-TerL complex.

Conclusions

In future, we would like to characterize all proteins implicated in packaging machinery of these phages. Moreover, it is important know the implication of these phages in mobilization and transference of virulence factors, codified in other MGE like SaPIs. That might lead to better adaptation of these strains in patients with pulmonary infections in a worldwide.

FEMS7-1249
Physiology / Biochemistry / Molecular Microbiology - Part III

REGULATION OF NITROGEN METABOLISM IN LACTOBACILLUS CASEI: THE SENSOR HISTIDINE KINASE PRCK IS A PHOSPHATASE

M. Zúñiga Cabrera[1], *B. Beamud Aranguren*[1], *A.B. García Lorenzo*[1], *C. Alcántara Baena*[1], *V. Monedero García*[1]
[1]*Instituto de Agroquímica y Tecnología de Alimentos, Biotecnología de Alimentos, Paterna, Spain*

Backgrounds

Lactic acid bacteria possess a limited amino acid biosynthetic capacity and need to obtain them from external sources. The response regulator PrcR plays a major role in the regulation of the proteolytic system in *Lactobacillus casei*. PrcR constitutes a signal transduction two-component system together with the sensor histidine kinase PrcK. PrcR regulates the expression of 353 genes in response to a complex source of amino acids. Gel mobility shift assays also showed that phosphorylation of PrcR enhanced its DNA binding activity.

Objectives

Characterization of the role of the PrcK in the regulation of the activity of PrcR.

Methods

A *L. casei* BL23 PrcK defective strain was obtained by a recombination strategy. Growth in media, acidification rate in milk and proteinase activity were determined. A comparative transcriptomic analysis was carried out by RNAseq. Phosphorylation of PrcR *in vivo* was monitored by PAGE with Phos-Tag and a specific anti-PrcR antibody.

Conclusions

Striking phenotypic differences were observed between the PrcR and the PrcK defective strains. In contrast to the PrcR mutant, the PrcK mutant did not display a growth defect in MRS, had low milk acidification rate and low proteinase activity. The transcriptomic analysis showed that 55 genes were differentially expressed compared to the parental strain and only 28 of them were also differentially expressed in the PrcR mutant strain. Analysis of the phosphorylation state of PrcR *in vivo* revealed that inactivation of PrcK resulted in the accumulation of phosphorylated PrcR indicating that PrcK acts as a phosphatase under our assay conditions.

FEMS7-0181
Special Event: From Research to Start-Up Creation

BIOSYSTEMS TECHNOLOGY LTD: A NEW UNIVERSITY OF EXETER SPIN OUT COMPANY

O. Champion[1], *R. Titball*[2]
[1]*University of Exeter, BioSystems Technology, Exeter, United Kingdom*
[2]*University of Exeter, BioSciences, Exeter, United Kingdom*

Backgrounds

Insect larvae *Galleria mellonella* can be used as a model host in microbiological, microbiota and toxin research and also as an ecotoxicological model organism and for drug development programmes. Results in *G. mellonella* correlate well with results in mammalian models and in research projects using *G. mellonella* the number of experimental vertebrates used has been reduced by up to 80%. The use of *G. mellonella* for R&D therefore reduces reliance on mammalian research models, which is aligned with European and UK policy, saves money and allows high throughput screening of compounds at a level that would not be possible using mammalian models.

Objectives

Having used *G. mellonella* for our research at the University of Exeter and after discussion with many members of the research community about the model host *G. mellonella*, we have identified the lack of standardisation as one of the key limitations of the model.

Methods

To over come this we have developed TruLarv™, research grade *G. mellonella* that are age and weight defined and they are surface decontaminated. TruLarv™ come from an in bred colony for which a whole genome sequencing programme is currently underway. Unlike bait shop waxworms TruLarv™ are not bred using antimicrobials or hormones.

Conclusions

Participation in Innovate UK's Innovation and Commercialising University Research (ICURe) programme in 2015 accelerated the commercial translation of this research and the University spin out company BioSystems Technology Ltd was founded for which private angel investment has been secured and the first product, TruLarv™, was launched in 2016.

FEMS7-1846
Special Event: From Research to Start-Up Creation

CARBOHYDRATE-MEDIATED DECONTAMINATION STRATEGIES AGAINST BIOTHREAT BACTERIA

M. Utratna[1], S. Deegan[1], K. Kubelková[2], J. Stulik[2], L. Joshi[3]
[1]Aquila Bioscience, Research and Innovation Building, Galway, Ireland
[2]Institute of Molecular Pathology, Faculty of Military Health Science- University of Defense, Hradec Kralove, Czech Republic
[3]Glycoscience Group,
National Centre for Biomedical Engineering Science- National University of Ireland Galway, Galway, Ireland

Backgrounds

Glycosylation is a key modification of proteins and lectin-carbohydrate interactions are essential in many host-microbial processes including adherence. The principle of using anti-adhesion strategies, based on glycobiology research, has already been shown to be effective in the prophylaxis and treatment, with examples for successful inhibition of bacteria, toxin and virus interactions.

Objectives

To study important carbohydrate-mediated microbial interactions, high-throughput lectin and glycan microarrays are increasingly utilised. We used custom made platforms covering a wide range of lectin specificities, carbohydrates presented on glycoproteins and neoglycoconjugates (NGCs) for profiling of biothreat agents (e.g. *Bacillus anthracis, Francisella tularensis, Clostridium botulinum*) and their low risk surrogate strains.

Methods

Initially, profiles of non-pathogenic surrogate microorganisms were used to nominate carbohydrate-based ligands for development and optimisation of decontamination technology against selected vegetative cells and spores. The technology was assembled and tested on non-pathogenic project models. Consequently, glycan/lectin binders were nominated by microarray technology for the selected biothreat bacteria. This novel technology, developed against BSL2 and BSL3 microorganisms, was tested in accredited labs for efficacy of decontamination and achieved high ability of capturing bacterial contaminants.

Conclusions

Overall, we describe a novel approach for studying lectin-carbohydrate interactions and the potential applications of glycomics microarrays in microbial research. Through a research-driven start-up company we present a prototype technology capable of capturing biothreat agents with high efficacy. Furthermore, this product captures viable bacteria which can be further analysed for diagnostic purposes. This innovative technology is portable, easy to use, non-toxic and environmentally friendly and will bring new insights for future decontamination strategies.

FEMS7-1694
Special Event: From Research to Start-Up Creation

IMPROVED BIOPROSPECTING: DARWIN'S WAY

C. Vilanova[1]
[1]*Darwin Bioprospecting Excellence SL, Biotechnology, Paterna, Spain*

Backgrounds

Four billion years ago, life appeared on Earth in the shape of bacterial communities. Since then, microorganisms have evolved to make a habitable planet and to harbor a fabulous diversity of metabolic pathways and chemical abilities.

Objectives

Biotechnology and Synthetic Biology yield a full range of powerful industrial solutions, but they rely on the identification, modification and use of biological parts already present in the wild. Improved bioprospecting is the best choice to provide bioengineers with new consortia, strains, genes or any other biological part.

Methods

Successful bioprospecting includes sampling unreported environments, massive use and development of new culture media, combining culture and non-culture approaches, and using artificial selection pressures towards to promote the selection of the desired biological variants.

Conclusions

By combining natural and artificial selection in improved bioprospecting, it is possible to identify biological parts from the huge repository that is our planet. Culture screening, multi-omics and selection-based approaches are central for improved bioprospecting.

FEMS7-3235
Taxonomy / Systematics

HIGH-QUALITY DRAFT GENOME SEQUENCE OF RAINEYA TEPIDIPHILA GEN. NOV., SP. NOV. A SLIGHTLY THERMOPHILIC BACTERIUM AND THE PROPOSAL OF RAINEYACEAE FAM. NOV.

L. Albuquerque[1], A.R. Polónia[2], H. Froufe[2], O. Lage[3], A. Lobo-da-Cunha[3], C. Egas[2], M. da Costa[1]
[1]Center for Neuroscience and Cell Biology- University of Coimbra, Laboratory of Microbiology, Coimbra, Portugal
[2]Biocant- Biotechnology Innovation Center, Next Generation Sequencing Unit, Cantanhede, Portugal
[3]Universidade do Porto,
CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Porto, Portugal

Backgrounds

One isolate designated, SPSPC-11^T, with an optimum growth temperature of about 45-50ºC and an optimum pH for growth between 7.5 and 8.0, was recovered from a reddish biofilm at the hot spring at São Pedro do Sul in Central Portugal (40° 46' N, 8° 4' W). Based on 16S rRNA gene sequence and phylogenetic analysis the new organism is most related to the species of the genus *Thermonema* with 16S rRNA gene pairwise sequence similarity of 82 to 83%.

Objectives

Growth, morphology, physiological and biochemical characteristics of the new isolate; determination of polar lipids, lipoquinones and fatty acid composition; determination of G+C content of DNA, 16S rRNA gene sequence and phylogenetic analyses; genome sequencing.

Methods

Cell morphology was examined by SEM and TEM. Phenotypic, chemotaxonomic and phylogenetic analysis were performed as described by Albuquerque *et al.*, 2014 (doi: 10.1016/j.syapm.2014.03.001). Genome was sequenced using paired-end sequencing and assembled with SPAdes (v 3.7.1).

Conclusions

Based on genotypic, phylogenetic, chemotaxonomic and phenotypic characteristics we describe a new species of a novel genus represented by strain SPSPC-11^T (=CECT 9012^T =LMG 29233^T) for which we propose the name *Raineya tepidiphila* gen. nov., sp. nov. We also propose the family *Raineyaceae* to accommodate this new genus.

FEMS7-0580
Taxonomy / Systematics

TAXONOMIC STUDY OF PHOTOBACTERIUM STRAINS ISOLATED FROM DISEASED FARMED FISH IN SOUTH SPAIN

D.R. Arahal[1], T. Lucena[1], A.M. Labella[2], M. Manchado[3], D. Castro[2], J.J. Borrego[2]
[1]Universitat de València, Microbiology and Ecology & Spanish Type Culture Collection CECT, Burjassot, Spain
[2]Universidad de Málaga, Microbiology, Malaga, Spain
[3]IFAPA- Junta de Andalucía, Centro El Toruño, Puerto de Santa Maria, Spain

Backgrounds

Strains H01100409B, H01100413B, and H27100402H were isolated in 2004 from several internal organs of diseased redbanded seabream (*Pagrus auriga*) reared in Andalusia (IFAPA Centro El Toruño, Puerto de Santa María, Cádiz). Presumptive identification bases on partial 16S rRNA gene sequences clustered these three isolates within the genus *Photobacterium*.

Objectives

Phenotypic and phylogenetic characterization of strains H01100409B, H01100413B, and H27100402H in order to complete their identification and formal taxonomic proposals according to the Bacteriological Code.

Methods

Phenotypic determinations included standard morphological, physiological, biochemical and chemotaxonomic methods commonly used in the taxonomy of *Vibrionaceae*.

The phylogenetic analysis was performed including a multilocus scheme (MLSA) using six housekeeping genes: *gapA* (glyceraldehyde-3-phosphate dehydrogenase A), *topA* (DNA topoisomerase I), *mreB* (cell wall structural complex MreBCD), *ftsZ* (GTP-binding tubulin-like cell division protein), *gyrB* (DNA gyrase B subunit), and 16S rRNA.

WGS Illumina Miseq platform was used for genome sequencing. Read analysis, de novo assembly and annotation was done with software included in Galaxy Orione and RAST servers. Average Nuclotide Indexes (ANIb, ANIm) were calculated with JSpecies and OrthoANI software and estimated DNA-DNA hybridization (eDDH) using the Genome to Genome Distance Calculator.

Conclusions

Based on the polyphasic study performed, we have determined that strains H01100409B (= CECT 9192) and H27100402H (= CECT 9190), represent a novel species each, for which the names *Photobacterium andalusiensis* sp. nov. and *Photobacterium malacitana*, respectively, are proposed. Strain H01100413B (= CECT 9191) is identified as a member of *Photobacterium aquimaris*.

FEMS7-0576
Taxonomy / Systematics

REAFFILIATION OF EDWARDSIELLA TARDA FISH ISOLATES TO THE NEW SPECIES EDWARDSIELLA PISCICIDA AND EDWARDSIELLA ANGUILLARUM

N. Buján[1], S. Balboa[1], J. L. Romalde[1], A. E. Estévez[1], B. Magariños[1]
[1]Fac. Bioloxía-CIBUS & Instituto de Acuicultura,
Microbioloxía e Parasitoloxía- Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Backgrounds

Edwardsiella is a genus belonging to the family *Enterobacteriaceae*, described in 1965 by Ewing and MacWhorter. Until 2012, the genus consisted of 3 species: *E. hoshinae*, *E. ictaluri* and *E. tarda*. Based on genetic studies two novel species, *E. piscicida* and *E. anguillarum* were described in 2013 and 2015 respectively. *E. piscicida* compiled only pathogenic strains isolated from fish showing phenotypic characteristics identical to *E. tarda*. The capacity to ferment mannitol and arabinose is the unique characteristic distinguishable for *E. anguillarum*, but is not enough to a *bona fide* identification

Objectives

The description of the new *Edwardsiella* species made necessary a deeper study of the phylogeny of the genus to clarify the position of isolates previously classified as *E. tarda*.

Methods

A total of 57 strains and 4 genomes retrieved from the GenBank database of *Edwarsiella* were used in this work. All strains were previously identified by classical phenotypical tests.
Seven gene loci were selected, including 16S rRNA gene, *adk*, *atp*D, *dna*J, *gln*A, *hsp*60 and *tuf*, to perform the MultiLocus Sequence Analysis (MLSA).
The OrthoANI values among the genomes were calculated using OAT (v0.93). Moreover, estimated DNA-DNA hybridization was determined by the genome-to-genome distance calculator (GGDC2.1).

Conclusions

The results indicate that *Edwardsiella* isolates obtained from fish and previously identified as *E. tarda* should be reclassified as *E. piscicida* or *E. anguillarum*. The scarce phenotypic differences among *E. tarda*, *E. piscicida* and *E. anguillarum* made necessary the use of molecular techniques to a correct identification of these species.

FEMS7-0985
Taxonomy / Systematics

THE NEW GENUS RODENTIBACTER INCLUDING RODENTIBACTER PNEUMOTROPICUS (PASTEURELLA PNEUMOTROPICA), RODENTIBACTER HEYLII AND SPECIES OF IMPORTANCE FOR IDENTIFICATION OF OPPORTUNISTIC PATHOGENS IN RODENT COLONIES

H. Christensen[1], *S. Adhikary*[1], *W. Nicklas*[2], *M. Bisgaard*[3], *R. Boot*[4], *T. Waberschek*[2], *B. Aalbæk*[1], *P. Kuhnert*[5], *B. Korczak*[5]
[1]*University of Copenhagen, Veterinary Animal Sciences, Frederiksberg C, Denmark*
[2]*Microbiological Diagnostics, German Cancer Research Centre, Heidelberg, Germany*
[3]*Bisgaard Consulting, Bisgaard Consulting, Viby Sj., Denmark*
[4]*Private, Private, Utrecht, The Netherlands*
[5]*Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland*

Backgrounds

It has been known for a long time that [*Pasteurella*] *pneumotropica* is not a member of *Pasteurella sensu stricto* and that biovars Jawetz and Heyl of [*Pasteurella*] *pneumotropica* should be classified as separate species.

Objectives

To investigate a collection of *Pasteurellaceae* mainly obtained from laboratory rodents, wild mice, and from mice and rats from petshops by extended pheno- and genotypic characterization and to classificy and name a new genus.

Methods

16S rRNA and partial *rpoB* gene sequencing were used to evaluate characters used for phenotypic identification and separation of taxa. Selected strains were further characterized based on whole genome comparison.

Conclusions

Whole genomic comparison of selected strains allowed the estimation of DNA-DNA renaturation and indicated the presence of 8 species within the genus. The type species is *R. pneumotropicus* including strains mainly from mice and including part of strains previously classified with [*Pasteurella*] *pneumotropica*. *Rodentibacter heylii* was proposed for a group that included the biovar Heyl of [*Pasteurella*] *pneumotropica*. *Rodentibacter ratti* which included the taxon 22 of Bisgaard was proposed for a new very large species mainly isolated from rats. *Rodentibacter heidelbergensis* and *Rodentibacter trehalosifermentans* also included strains only from rats. Strains from *Myodes glareolus* (bank vole) (taxon 41 of Bisgaard) were proposed as *Rodentibacter myodis*, two strains from rats including the reference strain of taxon 17 of Bisgaard as *Rodentibacter rarus* and another group with strains from *Apodemus* spp. were proposed as the new species *Rodentibacter mrazii*. The taxonomic changes will improve the identification of members of *Pasteurellaceae* isolated from rodents.

FEMS7-0608
Taxonomy / Systematics

COMPARATIVE GENOMIC FEATURES OF BACILLUS VELEZENSIS, BACILLUS AMYLOLIQUEFACIENS AND BACILLUS SIAMENSIS

B.H. Chun[1], K.H. Kim[1], S.E. Jeon[1], C.O. Jeon[1]
[1]Chung-Ang University, Department of Life Science, Seoul, Republic of Korea

Backgrounds

Three *Bacillus* groups (*B*. *velezensis*, *B*. *amyloliquefaciens*, and *B*. *siamensis*) are not differentiated based on 16S rRNA gene sequences because they are very closely related each other. Therefore, using pan-genomes of the three *Bacillus* groups we investigated the phylogenetic relatedness and compared their genomic and metabolic features.

Objectives

The aim of this study is to investigate the comparative genomic and metabolic features of three *Bacillus* groups with high phylogenetic relatedness.

Methods

Genomes that were possibly affiliated to the three *Bacillus* groups were retrieved from GenBank based on >98% of 16S rRNA gene sequence similarities and >95% of average nucleotide identity values to their respective type strains. After removing low quality genomes using the CheckM program, the comparative genomic and metabolic features of three *Bacillus* groups were investigated using pan-genome.

Conclusions

The 64 high quality genomes of *B*. *velezensis* (54), *B*. *amyloliquefaciens* (6), and *B*. *siamensis* (4) were selected and A phylogenetic analysis based on 16S rRNA gene sequences showed that the three *Bacillus* groups were undifferentiated phylogenetically, while a phylogenetic analysis using 1,957 core genes showed that they were clearly differentiated into three different phylogenetic lineages. The three *Bacillus* groups had similar COG distribution patterns, but their molecular phenotype-based relatedness was differentiated into three groups. The comparative genome analysis of three *Bacillus* groups showed that all *B*. *velezensis* strains harbor a macrolactin gene cluster, while all *B*. *siamensis* strains have a xanthine metabolic gene cluster.

FEMS7-2527
Taxonomy / Systematics

COMPLETE GENOME SEQUENCE OF A NEW SPECIES OF THE GENUS SALINIVIBRIO

R.R. de la Haba[1], *C. López-Hermoso*[1], *C. Sánchez-Porro*[1], *A. Ventosa*[1]
[1]*Universidad de Sevilla, Microbiology and Parasitology, Sevilla, Spain*

Backgrounds

During an environmental survey in order to study the biodiversity of different hypersaline habitats 70 strains phylogenetically affiliated to the genus *Salinivibrio* were isolated and characterized. A total of 34 out of the 70 isolates strains grouped into a different lineage clearly separated from the *Salinivibrio* named species and may constitute a new species of this genus.

Objectives

The aim of this study was to obtain and to analyze the complete genome sequence of the strain AL184, a representative member of this putative new species of *Salinivibrio*.

Methods

A combined sequencing approach was accomplished by using the Illumina and PacBio technologies. Raw data were subjected to quality control and the resulting subset was assembled using SPAdes v. 3.9.1 with specific options for hybrid assembly, besides other additional assemblers for comparative purposes. The resulting genome was annotated and further analyzed.

Conclusions

Sequencing depths of 1000X and 350X using Illumina and PacBio technologies, respectively, was enough to completely reconstruct the 3.4 Mb genome of the strain AL184. SPAdes assembler produced the best results allowing to recover a single contig sequence. This complete genome can be used as a reference for future genome assemblies in the genus *Salinivibrio* as well as to study the synteny of other draft *Salinivibrio* genomes.

FEMS7-1809
Taxonomy / Systematics

THE FAMILY ISOSPHAERACEAE: NEW INSIGHTS FROM COMPARATIVE GENOMICS

S. Dedysh[1], A. Ivanova[1], D. Naumoff[1], W. Liesack[2]
[1]Winogradsky Institute of Microbiology- Research Center of Biotechnology RAS, Microbiology of Wetlands, Moscow, Russia
[2]Max Planck Institute for Terrestrial Microbiology, Biogeochemistry, Marburg, Germany

Backgrounds

The family *Isosphaeraceae* accommodates stalk-free planctomycetes with spherical cells, which can be assembled in short chains, filaments or aggregates. These bacteria inhabit a wide variety of terrestrial and aquatic habitats. Their roles in nature, however, remain elusive due to difficulties of characterizing these bacteria by means of traditional cultivation approaches.

Objectives

This study was undertaken to determine the genome sequence of *Paludisphaera borealis* PX4^T and to compare it to those of other *Isosphaeraceae* members, for which complete genomes are presently available. *P. borealis* PX4^T is a newly described member of the *Isosphaeraceae* from boreal peatlands.

Methods

Genome sequencing was performed using the PacBio RSII platform. Annotation with *PROKKA* package was performed against the *UNIPROT* database and manually constructed database of annotated planctomycete genomes.

Conclusions

The finished genome of *Paludisphaera borealis* PX4^T consists of a 7.5 Mb chromosome and two plasmids, 112 and 43 kb in size. The genome size in other family members varies from 5.53 Mb in *Isosphaera pallida* IS1B^T to 9.74 Mb in *Singulisphaera acidiphila* DSM18658^T. All analyzed *Isosphaeraceae* planctomycetes have plasmids in numbers varying from one to four. The two plasmids from *P. borealis* display synteny to plasmids from other family members, suggesting their common evolutionary origin. Genomes of mesophilic *Isosphaeraceae* planctomycetes encode a large variety of carbohydrate-active enzymes, providing the potential to utilize a wide range of carbohydrates and glycoconjugates. In summary, our comparative genomic analysis revealed an extremely high glycolytic potential in *Isosphaeraceae* planctomycetes, which remains to be explored in future studies.

FEMS7-0484
Taxonomy / Systematics

ENTOMOPATHOGEN ID: A MULTI-LOCUS SEQUENCE ALIGNMENT RESOURCE FOR ENTOMOPATHOGENIC FUNGI

C. Dunlap[1], *J. Ramirez*[1], *D. Labeda*[2]
[1]*NCAUR, Crop Bioprotection, Peoria, USA*
[2]*NCAUR, Mycotoxin Prevention and Applied Microbiology research unit, Peoria, USA*

Backgrounds

The ability to correctly identify entomopathogenic fungi is an important step in developing biopesticides and effectively communicating research results. Over the years, identifying entomopathogenic fungi has evolved from a system based on diagnostic morphological and physiological characters to molecular DNA based systems. Internal transcribed spacer (ITS) sequences have become the barcode of choice when identifying unknown fungi. While ITS sequencing is a great tool for identifying unknown fungi, it often lacks sufficient resolution to accurately identify common entomopathogenic fungi. The problem is further compounded by not having a curated database of reference sequences based on type material to compare sequences against.

Objectives

Develop a web-based Multi-Locus Sequence Alignment resource for common Hypocrealean entomopathogenic fungi.

Methods

The Entomopathogen ID Multi-Locus Sequence Alignment resource is a curated sequence database built on sequences from MLSA and other taxonomic studies of common Hypocrealean entomopathogenic fungi spanning the taxonomic families of Cordycipitaceae, Clavicipitaceae and Ophiocordycipitaceae.

Conclusions

The database provides a new resource to improve the taxonomic characterization of this group of fungi.

FEMS7-2345
Taxonomy / Systematics

ASSESSING THE GLOBAL DIVERSITY OF A GENUS USING PHYLOGENOMICS

M. Garcia-Garcera[1], *M. Touchon*[1], *S. Brisse*[1], *E.P. Rocha*[1]
[1]*Institut Pasteur, Genomique Evolutive des Microbes, Paris, France*

Backgrounds

Most bacteria have poorly characterized environmental reservoirs and unknown closely-related species. This hampers the study of bacterial evolutionary ecology because both the environment and the genetic background of ancestral lineages are unknown.

Objectives

Our objectives are to assess the global diversity of a genus and its environmental distribution.

Methods

We combined metagenomics, comparative genomics, and phylogenomics to overcome this limitation, to identify known and novel taxa, and to propose environments where they can be isolated. We applied this method to characterize the ecological distribution of known and novel lineages of *Acinetobacter* spp.

Conclusions

We observed two major environmental transitions at deep phylogenetic levels, splitting the genus into three ecologically differentiated clades. One of these has rapidly shifted towards host-association by acquiring genes involved in bacteria-eukaryote interactions. We show that environmental perturbations affect species distribution in predictable ways*:* administration of antibiotics to bovines produces shifts from diverse to highly uniform, human-associated, communities of *Acinetobacter* spp. Our results uncover the diversity of bacterial lineages, overpassing the limitations of classical cultivation methods, and highlight the role of the environment in their evolutionary history

FEMS7-1210
Taxonomy / Systematics

SPECIES STATUS OF THE PSEUDOMONAS STUTZERI GENOMOVARS

E. García-Valdés Pukkits[1], *G. Margarita*[2], *M. Magdalena*[2], *A. Busquets*[2], *J. Lalucat*[1]
[1]*University of the Balearic Islands, Biology- Microbiology and IMEDEA, Palma de Mallorca, Spain*
[2]*University of the Balearic Islands, Biology- Microbiology, Palma de Mallorca, Spain*

Backgrounds

Pseudomonas stutzeri was first described by Burri and Stutzer (1895) and van Niel and Allen (1952) precisely defined its phenotypic features and discussed its definitive designation as *P. stutzeri* by Lehmann and Neumann. *It* is an extremely ubiquitous species found in soils and marine and fresh waters, but it is also isolated as opportunistic pathogen in clinical specimens. In 1995 Ursing and collaborators coined for *P. stutzeri* the concept of genomovar as “genomic groups of strains delimitated by DNA-DNA pairing that are phenotypically so similar that they cannot be differentiated for the time being”. Nowadays 22 genomovars have been described within the species and a reference strain has been proposed for each genomovar.

Objectives

Comparative genomics of all the genomovar reference strains will confirm the experimental genomovar delineation and also clarify the genomic similarities and differences among *P. stutzeri* genomovars. Whole-cell MALDI-TOF MS clearly allows the differentiation of *P. stutzeri* genomovars. Combination of both techniques will sustain the proposal of a new bacterial species for each genomovar.

Methods

Whole genome sequences of 83 *P. stutzeri* strains, including all genomovar reference strains and distinct species belonging to the *P. stutzeri* group were analysed. ANI, GGDC, SpecI, as well as core and pangenome analyses were performed.

Conclusions

The genome molecular data obtained were in accordance with the MALDI-TOF MS results and clearly confirmed that at least 11 genomovars with more than one strain each, should be proposed as new species within the genus *Pseudomonas*.

FEMS7-1113
Taxonomy / Systematics

TAXONOMIC CLARIFICATION OF THE PSEUDOMONAS SYRINGAE SPECIES GROUP STATUS BY PHYLOGENOMIC ANALYSIS

M. Gomila[1], *A. Busquets*[1], *M. Mulet*[1], *E. García-Valdés*[1], *J. Lalucat*[1]
[1]*University of the Balearic Islands, Biology, Palma de Mallorca, Spain*

Backgrounds

The genus *Pseudomonas* is taxonomically divided in two phylogenetic lineages (*P. aeruginosa* and *P. fluorescens*) based on the multilocus sequence analysis (MLSA) of four housekeeping genes. The *P. fluorescens* lineage contains six groups, one of them represented by *P. syringae*. Gardan and colleagues established in this species eight genomic groups, called genomospecies, based on DNA–DNA hybridization (DDH) analysis. Some of them later on were reclassified as different taxonomic species (*P. amygdali*, *P. avellanae, "P. coronafaciens"*, *P. ficuserectae*, *P. meliae, P. savastanoi*, *P. syringae*, *P. tremae*, and *P. viridiflava*). The taxonomic situation is more confuse when the pathovars are considered. Currently, the *P. syringae* species group is subdivided into over 60 pathovars defined by pathogenic characters, nine genomospecies defined by DDH and 13 phylogenetic groups (phylogroups) defined by MLSA.

Objectives

The main objective is to clarify the taxonomic delineation of *P. syringae* species group based on the phylogenomic analysis of more than 100 genomes sequenced and available in the database assigned to this group.

Methods

MLSA, Average nucleotide identity based on BLAST (ANIb) and Mummer (ANIm), Genome-to-Genome Distance Calculator (GGDC) as well as core and pangenome analyses have been performed to delineate the genomic species.

Conclusions

The analysis allowed to infer the taxonomic affiliation of all genomes analysed, some of them not correctly assigned. The pathovars did not follow the genomic clusters already defined. New genomic groups can be distinguished belonging to putative novel species. Genomic and phylogenetic approaches will provide the basis for a more reliable demarcation of *Pseudomonas* phytopathogenic species.

FEMS7-2201
Taxonomy / Systematics

CITROBACTER LUSITANIAE SP. NOV. AND CITROBACTER VIEIRENSIS SP. NOV.: TWO NOVEL CITROBACTER SPECIES ISOLATED FROM AQUATIC SAMPLES

T. Gonçalves Ribeiro[1], B. Ribeiro Gonçalves[2], M. Silva[2], Â. Novais[1], E. Machado[1,3], L. Peixe[1]
[1]Faculty of Pharmacy- University of Porto, Microbiology, Porto, Portugal
[2]Institute of Molecular Medicine- Faculty of Medicine- University of Lisbon, Microbiology, Lisboa, Portugal
[3]Faculty of Health Sciences- University Fernando Pessoa, Microbiology, Porto, Portugal

Backgrounds

The relevance of *Citrobacter*, a genus comprising opportunist pathogens, is well known as it could act as a source of antibiotic resistance genes (*qnrB* and *bla*$_{CMY-2}$). Nevertheless, *Citrobacter* species differentiation based on conventional tests is problematic, preventing the recognition of species with greater clinical significance. Recently, we demonstrated that *recN* (DNA repair protein) based phylogenetic analysis provides an accurate discrimination among *Citrobacter* species, and unveiled isolates not affiliated to previously recognized species.

Objectives

The purpose of this work was to define the taxonomic position of two representative strains belonging to two potential novel *Citrobacter* species, using genotypic and phenotypic approaches.

Methods

Two strains, A60^T recovered from a water well and A316^T from a natural spring, both collected in Portugal, were characterized. These strains were sequenced using Illumina MiSeq. Draft genomes were obtained using SPADes, and annotated by Prokka software. The similarity of 16S rRNA gene sequence of strains A60^T and A316^T to those of other *Citrobacter* species type strains were <98.9% and <99.0%, respectively. Phylogenetic analysis based on *recN* sequences showed a clear distinction of A60^T or A316^T and the type strains of the closest related *Citrobacter* species, with A60^T clustering with clinical isolates deposited in public databases and A316^T in one branch. Genomic similarity assessed by Average Nucleotide Identity (ANI) between A60^T or A316^T and type strains of closely related *Citrobacter* species (*C. freundii, C. europaeus* and *C. braakii*) was in all cases lower than 94.5%. The ability to metabolize different compounds further discriminated A60^T or A316^T from other *Citrobacter* species. The G+C content of strain A60^T and A316^T is 52.0% and 51.5%, respectively.

Conclusions

The results obtained support the description of two novel species of the genus *Citrobacter*, for which the names *Citrobacter lusitaneae* sp. nov. (A60^T as type strain) and *Citrobacter vieirensis* sp. nov. (A316^T as type strain) are proposed.

FEMS7-0600
Taxonomy / Systematics

POLYSACCHARIDE DECOMPOSERS AMONG HALOPHILIC ARCHAEA - A SYSTEMATIC GENOMIC AND PHENOTYPIC APPROACH

R.L. Hahnke[1], *J.P. Meier-Kolthoff*[1], *M. Göker*[1], *B.J. Tindall*[1]
[1]*DSMZ, Microorganisms, Braunschweig, Germany*

Backgrounds

Some *Bacteria* and *Archaea* taxa are known to thrive in carbohydrate-rich habitats as decomposers of particulate organic matter. In contrast, few polysaccharides have been tested and few taxa have been systematically investigated for polysaccharide degradation.

Objectives

With the aim of obtaining deeper insights into the evolution of carbohydrate metabolism in *Halobacteria*, we tested type strains in the family *Natrialbaceae* (*Archaea*) for their potential and ability to decompose polysaccharides.

Methods

Strains were incubated in media with NaCl concentrations of 10–32%, low nutrient concentrations and azurin-crosslinked polysaccharides in microtiter plates. Genome-scale phylogenies were inferred from whole proteomes using the Genome BLAST Distance Phylogeny (GBDP) approach. Genes encoding carbohydrate active enzymes were retrieved from the genome by matching the CAZy database.

Conclusions

Strains belonging to the genera *Halopiger* and *Halostagnicola* were rich in CAZymes, but strains belonging to the genera *Natronococcus*, *Natrinema*, *Natronorubrum*, *Natronolimnobius*, *Natronobacterium*, *Halobiforma* and *Halovivax* were not. Surprisingly, the diversity of polysaccharide decomposition differed significantly within the genera *Haloterrigena* and *Natrialba*. Galactomannan, pachyman, Barley-β-glucan and CM-cellulose were hydrolyzed only above 23% NaCl. Furthermore, the ability to grow on polysaccharides is directly correlated to the presence of genes of the semi-phosphorylated Entner-Doudoroff pathway in their genomes.

The results presented here demonstrate the advantage of a combined genomic and phenotypic approach to the systematic high-throughput screening of polysaccharide decomposition in prokaryotic taxonomy. Furthermore, the results provide additional information for the description and classification of microbial species and led to the discovery of so far hidden physiological features.

FEMS7-1739
Taxonomy / Systematics

DIVERSITY OF HALOPHILIC ACTINOBACTERIA FROM ARID REGIONS OF SAUDI ARABIA FOR POTENTIAL APPLICATION IN THE PRODUCTION OF COMMERCIAL ENZYMES

W. Hozzein[1], S. Alharbi[2], A. Chinnathambi[2]
[1]King Saud University, College of Science, Riyadh, Saudi Arabia
[2]King Saud University, College of Science- Microbiology Department, Riyadh, Saudi Arabia

Backgrounds

Enzymes of halophilic microorganisms have many industrial advantages over their non-halophilic counterparts. The halophilic actinobacteria from the Saudi habitats have been largely unexplored and it is of great interest to study them for their potential biotechnological applications.

Objectives

The main objectives of the present study were to isolate halophilic actinobacteria from the arid regions of Saudi Arabia, study their diversity, screen them for their enzymatic potential and identify them to the species level by the polyphasic approach.

Methods

104 soil samples representing different desert and extreme arid habitats in four different regions in Saudi Arabia were collected. The elective isolation of 465 halophilic actinobacterial strains was carried out using different selective media. After de-replication of the isolates, 329 dissimilar halophilic strains were obtained. The diversity of the isolated organisms was estimated phylogenetically by analyzing the 16S rRNA gene sequences. The actinobacterial isolates were then screened for enzymatic activities, namely, amylase, cellulase, lipase and protease. The enzymatically-active strains and the interesting organisms from the biodiversity have been identified by studying their morphological and chemotaxonomical characteristics and confirmed by phylogenetic analysis.

Conclusions

The results revealed that the isolates belong to 17 families and 27 genera. The enzymatic activities rate was found to be 74%, 26%, 34% and 55% for amylase, cellulase, lipase and protease production, respectively, which may be promising commercially. The taxonomic results revealed that many strains will be described as new genera and species. The results are very encouraging and Saudi habitats could be considered as a good source for potentially useful actinobacteria.

FEMS7-3068
Taxonomy / Systematics

IDENTIFICATION AND CHARACTERIZATION OF DERMABACTER JINJUENSIS SP. NOV. AS A CLINICAL PATHOGEN RESOURCE IN KOREA

K.J. Hwang[1], K.M. Lee[1], M.H. Kim[1], Y.K. Park[1]
[1]Koera National Institute of Health, Pathogen Resource TF, Cheongju-si, Republic of Korea

Backgrounds

Species-level identification is crucial to characterize unidentified bacterial pathogens for the efficient development and management of bacterial resources. Unidentified strains were collected from branch banks of the National Culture Collection for Pathogens (NCCP).

Objectives

A total of 437 unidentified pathogens based on the results of the VITEK 2 system were identified using 16S rRNA gene analysis and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the development of national resources in NCCP. On the basis of phenotypic, biochemical, chemotaxonomic, and molecular studies, we reported that the 32T isolate from a clinical patient showed new species of *Dermabacter* in Korea.

Methods

Phylogenetic analysis based on 16S rRNA gene sequences revealed that 32T belonged to the genus *Dermabacter* and was closely related to *Dermabacter hominis* DSM 7083T (98.34%). And 32T was considered a potentially novel species because of its low score value (1.886); MALDI-TOF MS identification scores of ≥1.7 but <2.0 are accepted for identification to the genus level. The major cellular fatty acids were anteiso-C17:0 (28.33%), C16:0 (24.05%), anteiso-C15:0 (17.83%), and iso-C16:0 (11.54%). The DNA G+C content of strain 32T was 63.16 mol%, and the DNA-DNA hybridization value between 32T and *D. hominis* DSM 7083T was 49±1.6%.

Conclusions

We propose strain 32T as the representative of a novel taxon, *Dermabacter jinjuensis* sp. nov., with the type strain DSM 101003T. The strain has been deposited at the NCCP (=NCCP 16133T) and is expected to be used as a valuable pathogen resource.

FEMS7-3002
Taxonomy / Systematics

CLASSIFICATION OF PSYCHROPHILIC GLIDING BACTERIA ISOLATED FROM ANTARCTICA

S. Králová[1], E. Staňková[1], I. Sedláček[1]
[1]Masaryk University, Department of Experimental Biology- Czech Collection of Microorganisms, Brno, Czech Republic

Backgrounds

Polar microbiology is a fast-growing field in the last years. As a part of polar systematics, the taxonomy of psychrophilic bacteria is focused on bacterial diversity in polar region, such as Antarctica.

Objectives

This study was focused on yellow pigmented and gliding Gram-stain negative bacteria isolated from the environment in Antarctica during years 2008-2014 (J. G. Mendel Station, James Ross Island). Aim of this study was to describe phylogenetic relationships of these psychrophilic bacteria against to already known species, as well as their physiology, biochemistry and other properties.

Methods

Isolated strains were firstly characterized with basic phenotyping including test for growth in different temperatures and in presence of NaCl, production of specific enzymes and ability to utilize different substrates. Secondly, all these strains were investigated by FAME (fatty acid methyl ester analysis), MALDI-TOF MS, repetitive PCR and their phylogenetic position was determined by partial 16S rDNA sequence analysis.

Conclusions

A group of 76 gliding psychrophilic bacteria was found among 587 pigmented strains in the present study. All strains were found to be Gram-stain negative, catalase positive rods with gliding motility enhanced by lower temperatures. All 76 strains were phylogenetically placed within the genus *Flavobacterium* forming at least 7 separated clusters. One strain was also Gram-stain negative and catalase positive psychrophilic bacterium with reduced gliding motility and expressing agarolytic activity. All these cold-adapted bacteria represent probably novel species within genus *Flavobacterium* and they might represent species with unique properties and/or producing cold-adaptive biomolecules applicable to biotechnology purposes.

FEMS7-0567
Taxonomy / Systematics

ARCOBACTER LEKITHOCHROUS SP. NOV., A NEW SPECIES ISOLATED FROM A MOLLUSCAN HATCHERY IN NORWAY

A. L. Diéguez[1], *S. Balboa*[1], *J. L. Romalde*[1]
[1]*CIBUS- Facultad de Biología. Universidade de Santiago de Compostela, Microbiologiay Parasitologia, Santiago de Compostela, Spain*

Backgrounds

The genus *Arcobacter* belongs to the family *Campylobacteraceae* and the type species is *Arcobacter nitrofogilis*, initially described as *Campylobacter* and reclassiffied as a new genus. *Arcobacter* shows a global distribution and a high diversity of ecological niches: marine environments, waste and drink water, animal faeces, associated to plants or in oil field communities among others.

Objectives

To characterize four strains: LFT 1.7^T (=CECT 8942^T= DSM 100870^T), LT2C 2.5, LT4C 2.8 and TM 4.6 (= CECT 8943= DSM 100869) isolated from larvae and seawater obtained during a study on the microbiota associated to Great scallop (*Pecten maximus*) in a hatchery in Bergen (Norway).

Methods

These isolates were fully characterized using phenotypic, genomic and chemotaxonomic approaches.

Conclusions

Multilocus Sequence Analysis (MLSA) of five housekeeping genes and the 16S rRNA gene placed the strains within the genus *Arcobacter* in a well differentiated branch with regard to the rest of the species. *In silico* hybridization as well as the Average Nucleotide Identity (ANI) analysis showed a low percentage of similarity with known species of *Arcobacter*. All isolates were Gram-negative and motile rods, oxidase and catalase positive and required sea salts for growth. The DNA G+C content was of 28.7 mol%. Strain LFT 1.7^T contained MK-6 as the sole respiratory quinone. Based on all of these characteristics, the four Norwegian isolates represent a new species within the genus *Arcobacter* for which the name *Arcobacter lekithochrous* sp. nov. is proposed, with strain LFT 1.7^T (=CECT 8942^T= DSM 100870^T) as the type strain.

FEMS7-1189
Taxonomy / Systematics

PHYLOGENOMIC ANALYSIS OF SPECIES IN THE PSEUDOMONAS PUTIDA PHYLOGENETIC GROUP

J. Lalucat[1], M. Gomila[2], A. Busquets[2], M. Mulet[2], E. García-Valdés[1]
[1]University of the Balearic Islands, Biology and IMEDEA CSIC-UIB, Palma de Mallorca, Spain
[2]University of the Balearic Islands, Biology, Palma de Mallorca, Spain

Backgrounds

Fifteen species have been recognized until December 2016 in the *Pseudomonas putida* phylogenetic group. Strains in the group are ecological relevant due to their metabolic versatility. However, also clinical strains of species in this group have been described. The whole genome sequence of all type strains in the group have been described and comparative genomics is for the moment the best approach to definitively identify the strains at the species level.

Objectives

Main objective is the taxonomic delineation of species in this phylogenetic group by using phylogenomic approaches. Hundred and thirty sequenced strains assigned to the genus *Pseudomonas*, or to species in the *P. putida* phylogenetic group, are analyzed to clarify their taxonomic position.

Methods

Four mainly accepted genomic tools in taxonomic analysis, namely Multilocus Sequence analysis (MLSA), Average Nucleotide Identity based on BLAST (ANIb), whole-genome based Average Nucleotide Identity (gANI) and Genome-to-Genome Distance Calculations (GGDC), are applied to 130 strains to delineate genomic species.

Conclusions

The 15 species type strains actually recognized within the group are clearly separated in the genomic analyses and at least 10 new genomic species can be delineated. Most of the putative new species are singletons, but others include several strains. A deep taxonomic analysis of each group is needed to propose formally the new species status.

FEMS7-1646
Taxonomy / Systematics

TAXONOMIC EVALUATION OF THE GENUS SALINIVIBRIO BASED ON MULTILOCUS SEQUENCE ANALYSIS AND OTHER MOLECULAR APPROACHES

C. Lopez-Hermoso[1], *R. Ruiz*[1], *T. Papke*[2], *C. Sanchez-Porro*[1], *A. Ventosa*[1]
[1]*Universidad de Sevilla, Microbiology and Parasitology, SEVILLA, Spain*
[2]*University of Connecticut, Molecular and Cell Biology, Stors, USA*

Backgrounds

The genus *Salinivibrio* includes halophilic bacteria and is commonly isolated from hypersaline habitats and salted food products. They grow optimally at ca. 7.5 % salts and are facultative anaerobic microorganisms. Currently, this genus comprises four species, one of them with three subspecies. Phylogenies based on the 16S rRNA gene sequence comparative analysis showed some incongruities, such as the clustering of the three subspecies as a polyphyletic group.

Objectives

The purpose of the present study was to refine the understanding of the phylogenetic relationships of the species and subspecies in the genus *Salinivibrio* and to clarify the current taxonomic status of the members of this genus by using Multilocus Sequence Analysis (MLSA) and DNA-DNA hybridization (DDH) approaches.

Methods

We have isolated and characterized 70 new strains from solar salterns located in different geographical locations. The MLSA study was based on the individual and concatenated sequence analysis of *gyrB*, *recA*, *rpoA* and *rpoD* housekeeping genes. The MLSA scheme was validated against DDH data by means of the Pearson's correlation coefficient.

Conclusions

These analyses permitted to include the isolated and type strains of *Salinivibrio* in four clearly different phylogroups, while one strain, represented by the type strain of the species *Salinivibrio sharmensis,* did not cluster with any of the other strains. Further the species level defined by a DDH value of 70 % was correlated with a 96 % cut-off for the concatenated MLSA gene sequence. Based on these criteria the phylogroup 1 could constitute a new separate species, while the strains included on the other three phylogroups are members of previously recognized *Salinivibrio* species.

FEMS7-1879
Taxonomy / Systematics

GENETIC DIFFERENTIATION OF BIOCONTROL RELATED TRICHODERMA STRAINS DEPOSITED AT THE SPANISH TYPE CULTURE COLLECTION (CECT) AS TRICHODERMA HARZIANUM

L. López-Ocaña[1], L. Moragues[2], A. Igual[1], P. Elizaquível[1], R. Aznar[1,2]
[1]University of Valencia, Spanish Type Culture Collection, Paterna Valencia, Spain
[2]University of Valencia, Microbiology and Ecology, Burjasot Valencia, Spain

Backgrounds

Trichoderma harzianum (Rifai, 1969) is a highly heterogeneous species that includes economically relevant strains effective in the control of soil-borne diseases. Due to the difficulty of separating the members of this genus most of the biocontrol strains were referred in the past to this species. Recently, a systematic revision on the *Trichoderma harzianum* complex revealed reorganization in the species composition, the description of new species and the re-identification of commercial biocontrol strains. Therefore, proper identification of strains at species -or even intra-species- level is advisable for commercial applications.

Objectives

To assess identification of strains deposited at the CECT as *T. harzianum* and to evaluate RAPD profiles and gene sequencing for intra-species differentiation.

Methods

A total of 35 *Trichoderma* reference strains, 31 *T. harzianum*, 2 *T. virens* and 2 *T. viride*, deposited at the CECT were examined through phylogenetic analyses with the nuc translation elongation factor 1-α (tef1 intron 4, TEF1), which proved efficient for species identification. RAPD profiles were determined with six different primers (OPA-2, 3, 10, 13 and 18 from Operon Technologic, and M13) for intra-species differentiation.

Conclusions

Analysis of TEF1 sequences revealed that strains deposited at the CECT as *T. harzianum* included members of other species in the *T. harzianum* complex, like *T. simmonsii*, *T. afroharzianum*, *T. guizhouense*, *T. atrobrunneum*, and other species like *T. citroviride*. Clustering analysis of combined RAPD profiles confirmed the species ascription of CECT *T. harzianum* strains obtained by TEF1 sequencing, and revealed slight differences within the species.

FEMS7-0587
Taxonomy / Systematics

PHOTOBACTERIUM TORUGNENSIS SP. NOV., A NEW BACTERIUM ISOLATED FROM DISEASED FARMED FISH

T. Lucena[1], D.R. Arahal[1], A.M. Labella[2], D. Castro[2], J.J. Borrego[2], M. Manchado[3]
[1]Universidad de Valencia, Microbiology and Ecology & Spanish Type Culture Collection, Paterna, Spain
[2]Universidad de Málaga, Microbiology, Malaga, Spain
[3]IFAPA - Junta de Andalucía, Centro El Toruño, Puerto de Santa María- Cádiz, Spain

Backgrounds

Strains H01100410B, H20040403B, and H20040405H were isolated from liver and spleen of captived redbanded seabream (*Pagrus auriga*) in a mortality outbreak in autumn 2011 at the Southatlantic coast of Spain (IFAPA Centro El Toruño, Puerto de Santa María, Cádiz). Presumptive identification bases on partial 16S rRNA gene sequences clustered these three isolates within the clade Phosphoreum in the genus *Photobacterium*.

Objectives

Phenotypic and phylogenetic characterization of strains H01100410B, H20040403B, and H20040405H in order to complete their identification. Formal taxonomic proposal according to the Bacteriological Code.

Methods

Phenotypic determinations included standard morphological, physiological, biochemical and chemotaxonomic methods commonly used in the taxonomy of *Vibrionaceae*.

The phylogenetic analysis was performed including a multilocus scheme (MLSA) using six housekeeping genes: *gapA* (glyceraldehyde-3-phosphate dehydrogenase A), *topA* (DNA topoisomerase I), *mreB* (cell wall structural complex MreBCD), *ftsZ* (GTP-binding tubulin-like cell division protein), *gyrB* (DNA gyrase B subunit), and 16S rRNA.

WGS Illumina Miseq platform was used for genome sequencing. Read analysis, de novo assembly and annotation was done with software included in Galaxy Orione and RAST servers. Average Nuclotide Indexes (ANIb, ANIm) were calculated with JSpecies and OrthoANI software and estimated DNA-DNA hybridization (eDDH) using the Genome to Genome Distance Calculator.

Conclusions

Based on the polyphasic study performed, we have determined that strains H01100410B, H20040403B, and H20040405H to be a new species, and we propose *Photobacterium torugnensis* sp. nov., with strain H01100410B (= CECT 9189) as type strain.

FEMS7-0966
Taxonomy / Systematics

VIBRIO RADICULARIS SP. NOV. A NEW VIBRIO SPECIES ASSOCIATED TO RHIZOSPHERE OF ARTHROCNEMUM MACROSTACHYUM

M. Maciá[1], M.A. Ruvira[1], M.J. Piñar[1], S. Navarro-Torre[2], E. Pajuelo[2], I.D. Rodriguez-Llorente[2], D. Arahal[1], M.J. Pujalte[1]
[1]Universidad de Valencia, Microbiology and Ecology & Spanish Type Culture Collection, Paterna, Spain
[2]Universidad de Sevilla, Departament of Microbiology and Parasitology, Sevilla, Spain

Backgrounds

Strain RA1 was isolated in February 2014 from the rhizosphere of *Arthrocnemum macrostachyum* sampled in the estuary of the river Odiel (Huelva, Spain). This strain was related to the genus *Vibrio* by 16S rRNA sequence comparison, being *Vibrio kanaloae* and *Vibrio toranzoniae* the closest relatives with 95.8 and 95.7 % sequence similarity in BLAST, respectively.

Objectives

Phenotypic and phylogenetic characterization of strain RA1.

Methods

Physiological and biochemical methods, including fatty acid analysis and phylogenetic analysis of the almost complete 16S rRNA sequence of strain RA1.

Conclusions

Strain RA1 has less than 97.0% similar to any other *Vibrio* species and is phylogenetically related to the Splendidus clade.

Cells are Gram negative, motile rods, chemoorganotrophic and facultatively anaerobic, able to ferment sugars. It is mesophilic and slightly halophilic, unable to grow without salts. Oxidase and catalase positive, able to reduce nitrate to nitrite. Glucose is fermented without gas production. Negative for Voges Proskauer and indol production. Positive for ADH but negative for LDC and ODC. Hydrolyzes DNA, casein and gelatin but not Tween 80 and alginate. Positive in API ZYM for alkaline and acidic phosphatases leucine arylamidase and trypsin activities. Growth on synthetic medium (BMA) plus sole carbon and energy source occurs with monosaccharides, organic acids and aminoacids.

Main FAME are C16:1ω7c/ C16:1ω6c (SF3), C16:0 and C18:1ω7c, as other *Vibrio* species.

Phenotypic and phylogenetic data confirm that RA1 represents a new species in the genus *Vibrio*, for which we propose the name *V. radicularis* sp. nov. with strain RA1T (CECT 9082T, LMG 29974T) as the type strain.

FEMS7-1318
Taxonomy / Systematics

ROSEOVARIUS SP. NOV. - A BACTERIUM ISOLATED FROM A SALINE SOIL BY DILUTION-TO-EXTINCTION CULTIVATION

D.J. Castro[1], I.M. Cerezo[1], M. Rodríguez[1], V. Béjar[1], F. Martínez-Checa[1]
[1]Facultad de Farmacia- Universidad de Granada, Microbiología, Granada, Spain

Backgrounds

The genus *Roseovarius* was first proposed with the description of *Roseovarius tolerans* as the type species of the genus within the family *Rhodobacteraceae.* This genus consists of 21 recognized species.

In this study we describe the strain D15^T, isolated from a sample of soil taken from Rambla Salada (Murcia) south-east Spain, a hypersaline rambla (a steep-sided river bed, normally dry but subject to flash flooding) using the dilution-to-extinction method.**Objectives**

The aim of this study was the characterization of the strain D15^T by a polyphasic approach, including phenotypic tests and genetic, chemotaxonomic and phylogenetic analyses.

Methods

Phenotypic characterization was carried out by morphological, physiological and biochemical test as API 20NE, API 50CH and API ZYM strips. Optimum growth conditions were determined by growing the strain in R2A medium at different NaCl concentration, pH and temperatures.

Conclusions

Cells were motile with a polar flagellum, rod-shaped, Gram-stain-negative, catalase- and oxidase-positive. They can grow optimally at 5% (w/v) NaCl, 30ºC and pH 7.0. The DNA G+C content of strain D15^T, estimated from the midpoint value (Tm) of their DNA was 59.2 mol%.

Phylogenetic analyses based on the 16S rRNA using the EzTaxon server showed that the most closely phylogenetically related species was *Roseovarius tolerans* with a similarity value of 96.12%.

The respiratory quinone (Q-10) and the fatty acid profiles of the strain D15^T were similar with those described for species of the genus *Roseovarius.*

Accordingly, on the basis of differences in phenotypic, chemotaxonomic characteristics and genetic distinctiveness, strain D15^T should be recognized as representing a novel species of the genus *Roseovarius.*

FEMS7-1330
Taxonomy / Systematics

MARINOBACTERIUM SP. NOV. - A BACTERIUM ISOLATED FROM A HYPERSALINE RAMBLA LOCATED IN MURCIA, SOUTH-EAST SPAIN

D.J. Castro[1], I.M. Cerezo[1], M. Rodríguez[1], V. Béjar[1], F. Martínez-Checa[1]
[1]Facultad de Farmacia- Universidad de Granada, Microbiología, Granada, Spain

Backgrounds

The genus *Marinobacterium*, belonging to the family *Alteromonadaceae,* within the class *Gammaproteobacteria,* was proposed by González *et al.* (1997). Currently, the genus comprises 16 strains with validly published names, isolated from different environments, such as a marine pulp mill, tidal flats, seawater, marine sediment, corals, roots and coastal seawater.

Objectives

In this study, we describe, by a polyphasic approach, a novel strain designated D7^T, isolated from a soil sample taken from a hypersaline rambla (a steep-sided river) located in Murcia, south-east Spain (Rambla Salada).

Methods

For the isolation of the strain D7^T we used the dilution-to-extinction method. Phenotypic characterization was carried out by morphological, physiological and biochemical test as API 20NE, API 50CH and API ZYM strips.

Conclusions

The cells were catalase- and oxidase-positive, Gram-stain-negative rods. Growth occurred at 15-40 °C (optimum 30-37°C), pH 5.5-9.5 (optimum pH 6.5-7.5) and with 0-7.5 % (w/v) NaCl (optimum 1-3 %).

Chemotaxonomic analysis showed that the respiratory quinone (ubiquinone-8) and fatty acid profiles were very similar to those described for species of genus *Marinobacterium*, including $C_{16:0}$ (28.7 %), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 26.6 %) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$*, 25.2%*). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain D7^T was the most closely related to the genus *Marinobacterium*, sharing the highest 16S rRNA gene sequence similarity of 98% with *Marinobacterium zhoushanense* and 95.7% with *Marinobacterium lutimaris*. The genomic DNA G+C content of the strain D7^T was 60.3 mol%.

On the basis of the phenotypic, chemotaxonomic and genotypic characteristics, we suggest that strain D7^T represents a novel species of the genus *Marinobacterium*.

FEMS7-1329
Taxonomy / Systematics

MULTILOCUS PHYLOGENETIC ANALYSIS OF THE GENUS LISTERIA: A PRAGMATIC TOOL FOR SPECIES IDENTIFICATION OF NEW ISOLATES

A. MARTINEZ-MURCIA[1], *A. Navarro*[2], *P. Elizaquivel*[3], *R. Aznar*[3]
[1]*Universidad Miguel Hernandez and Genetic PCR Solutions TM, Microbiologia, Orihuela, Spain*
[2]*Genetic PCR Solutions TM, Research, Elche, Spain*
[3]*Universitat de València, Microbiología y Ecología - Colección Española de Cultivos Tipo CECT, Valencia, Spain*

Backgrounds

According to the List of Prokaryotic names with Standing in Nomenclature (LPSN; www.bacterio.net/listeria.html), 19 species and 4 subspecies have been described for the genus *Listeria*. While *L. ivanovii* pathogenicity seems limited to ruminants, *L. monocytogenes* is the causative agent of listeriosis, a serious zoonotic disease with dramatic consequences in humans. Conventional microbiological standard methods are indispensable for the isolation of *Listeria*. Further, species identification by using biochemical tests may not give enough resolution due to their overlapping phenotypic diversity. Several phylogenies based on housekeeping gene sequencing have greatly improved the ability to discriminate *Listeria* species. To our knowledge, however, a comprehensive multi-gene phylogenetic approach is not available.

Objectives

To carry out a Multi-Locus Phylogenetic Analysis (MLPA) for all *Listeria* species described to date, using type strains and other “bona-fide” material acquired from public culture collections furtherly identified by 16S rDNA sequencing.

Methods

MLPA selected housekeeping gene-fragments were: *gyrB* (524bp), *rpoB* (592bp), *recA* (555bp), *gyrA* (783bp), *atpA* (611bp), *parE* (539bp), and *cpn60* (830bp).

Conclusions

Overall, single-gen phylogenetic relationships agreed to each other suggesting these genes evolve in concert and represent a reasonable phylogenetic hypothesis. Species clustering showed in the concatenated MLPA-tree (3.848 bp) was highly reproducible as indicated by bootstrap values (ca. 100%). The pictures showed a number of complex clades, some of them relatively distant to this referred as “*Listeria* sensu stricto”. High intra-species diversity in *L. monocytogenes* was ascertained. The MLPA approach performs as a robust frame to afford *Listeria* diversity for species identification.

European Union [FP7/2007-2011] Aquavalens-Grant311846

FEMS7-0459
Taxonomy / Systematics

THE WHOLE-GENOME ANALYSIS OF SOME EPIPHYTIC AND ASSOCIATIVE ISOLATES OF HETEROCYSTOUS CYANOBACTERIUM ANABAENA VARIABILIS

L. Mikheeva[1], *P. Meshcheryakova*[1], *E. Karbysheva*[1], *O. Zhenavchuk*[1]
[1]*Lomonosov Moscow State University, Biological faculty, Moscow, Russia*

Backgrounds

Filamentous heterocyst-forming cyanobacterium *Anabaena variabilis* ATCC 29413 is well investigated model organism with completely sequenced genome. Our experimental results and the data of other authors revealed the remarkable similarities of molecular-genetic properties between *A. variabilis* ATCC 29413, epiphytic strain *Anabaena* sp.182 and several strains of culturable minor cyanobionts from *Azolla* Lam. with genetic nature unresolved up to date.

Objectives

The aim of this work was the whole-genome sequencing of two *Anabaena* strains: rice plant epiphytic strain *Anabaena* sp.182 from Vietnam and the strain *Anabaena* sp.V5, culturable minor cyanobiont from leaf cavities of *Azolla pinnata*.

Methods

Pyrosequencing analysis of the entire *Anabaena* sp.182 and *Anabaena* sp.V5 genomes was performed using Genome Sequencer (GS) FLX with *A. variabilis* ATCC 29413 as a reference genome.

Conclusions

The obtained sequencing data have allowed us to establish the exact genetic nature of investigated strains, and to classify them as very closely related variants of model *A. variabilis.* Comparing to *A. variabilis* ATCC 29413 (originally isolated in 1964 in Missisippi) the *Anabaena* sp.V5 genome is characterized only by the loss of linear incision element (37 kb). In the genome of *Anabaena* sp.182 there is 1.4 kb insertion in gene *Ava_2562,* coding the hybrid response regulator protein that participates in Ca^{2+}-promoted regulation of phycobiline degradation, exopolysaccharide and gormogonia formation processes.

Such minimal genetic polymorphism of independent cyanobacterial isolates reveals the significant genome stability and adaptive capacity of *A. variabilis* that provide its wide spread occurrence and the ability to form stable associative complexes with plants due to mixotrophic metabolism.

FEMS7-2136
Taxonomy / Systematics

QUICKLY GROUPING PROKARYOTIC GENOMES INTO PHYLOGENETICALLY COHERENT GROUPS: SAME-SPECIES RESOLUTION

G. Moreno-Hagelsieb[1], *S. Wu*[1]
[1]*Wilfrid Laurier University, Biology, Waterloo, Canada*

Backgrounds

Reported genomes do not always have a species names, and when they do, the phylogenetic "spread" of each species varies. Thus, there is a need for methods for grouping genomes into phylogenetically coherent groups. Available methods can be computationally intensive. DNA signatures are fast to compute and compare, and thus might be useful for species-level resolution of prokaryotic genomes.

Objectives

To test the similarity of clusters obtained using DNA signatures against those obtained using other phylogenomic distances, and 16S rRNA sequences.
To suggest thresholds using DNA signatures for species-level genome clustering equivalent to those obtained with more time-consuming thresholds.

Methods

We obtained the species names for each genome in the complete genomes dataset from NCBI. These species names were used to determine thresholds for species-level clustering using different phylogenomic-based, marker-gene-based, and compositional-based (DNA signatures calculated at the di, tri, and tetra-nucleotide levels) distances. We compared the clusters thus obtained by finding the corresponding clusters, defined as those containing the highest number of genomes in common. Their similarity was expressed as percent of genomes in common. Only cluster with ten genomes or more were used to calculate cluster similarity.

Conclusions

We found that the clusters obtained using DNA signatures are very similar to those obtained using other, more time-consuming distances. It is therefore adequate to save time in constructing these clusters using DNA signatures before attempting more complicated, computer-intensive, analyses.

FEMS7-3192
Taxonomy / Systematics

PASTEURELLACEAE - BACTERIAL DIVERSITY AND ADAPTATION IN WILDLIFE HOSTS

K. Mühldorfer[1]
[1]*Leibniz Institute for Zoo and Wildlife Research, Department of Wildlife Diseases, Berlin, Germany*

Backgrounds

The family of the Pasteurellaceae includes phenotypically and genetically highly diverse bacterial species that play different roles in human and animal health and diseases. The biodiversity of the Pasteurellaceae causes significant difficulties in diagnostic laboratories, impacting not only the success of bacterial isolation or genetic detection but also accurate identification and taxonomic classification. In recent years, diagnostic challenges become more obvious in the field of zoo and wildlife diseases, where the number of variant or unknown bacteria belonging to the family of Pasteurellaceae is constantly increasing.

Objectives

This study aims for a better understanding of the diversity of Pasteurellaceae bacteria in a wide variety of captive and free-ranging wildlife hosts.

Methods

A total of 175 Pasteurellaceae isolates collected from diseased and clinically healthy mammals, birds and reptiles were identified based on their primary phenotypic characteristics. Combined 16S rRNA and *rpoB* gene analyses were used to investigate their genetic relatedness and phylogenetic relationship to known bacterial taxa.

Conclusions

Genetic results provide clear evidence of highly diverse, unclassified or unknown Pasteurellaceae-like species in wildlife hosts. For several isolates collected from small mammals, marsupials, ungulates, carnivores, birds and reptiles, the level of identification did not reach a specific bacterial genus or bacterial species. Furthermore, phylogenetic analyses revealed individual bacterial lineages and distinct clusters, which seem to correspond to a specific host or systematic group of animals and may indicate specific bacteria-host relationships.

FEMS7-1107
Taxonomy / Systematics

A PROTEIN SEQUENCE SET FOR MULTILOCUS SEQUENCE ANALYSIS (MLSA) AND PHYLOGENOMIC AFFILIATION IN THE MARINE ROSEOBACTER LINEAGE

B. Nogales[1], *M. Mas-Lladó*[2], *R. Bosch*[1]
[1]*University of the Balearic Islands UIB and IMEDEA UIB-CSIC, Microbiology, Palma de Mallorca, Spain*
[2]*University of the Balearic Islands, Microbiology, Palma de Mallorca, Spain*

Backgrounds

The Roseobacter lineage (*Rhodobacteraceae*, *Alphaproteobacteria*) is an important group of marine bacteria. Robust phylogenetic reconstructions using concatenates of a variable number (i.e. 52-78) of single-copy, conserved protein sequences have been done. This provides a backbone for the affiliation of new isolates. However, the pace of genome sequencing in this lineage requires continuous reevaluation of a *core* protein set useful for phylogeny.

Objectives

In this study we propose a set of fourteen protein sequences for MLSA in the Roseobacter lineage that reproduces correctly the phylogeny of the lineage and can be used for the affiliation of new isolates.

Methods

To define the MLSA protein set we calculated a *core* proteome using 96 Roseobacter genome sequences (114 proteins). We retrieved the conserved residues, concatenated them and calculated a phylogenetic tree by maximum parsimony. After calculating individual trees for each protein we selected fourteen of them (4,462 amino acid positions) whose concatenated sequences reproduced better the topology of the *core* proteome tree. To check the usefulness of this MLSA protein set we included 69 additional genome sequences and calculated a new tree (4,412 aligned positions).

Conclusions

We observed differences only in the grouping of two isolates belonging to the two most deeply-branching genomic groups and in the intra-group branching order of a third genomic group. Therefore, most of the isolates were placed in the expected genomic group and genus, confirming the usefulness of this approach for the rapid and robust affiliation of new isolates of the Roseobacter lineage.

FEMS7-0679
Taxonomy / Systematics

ARCOBACTER LACUS SP. NOV. AND ARCOBACTER CAENI SP. NOV., TWO NEW SPECIES ISOLATED FROM RECLAIMED WATER

A. Pérez-Cataluña[1], *M.J. Figueras*[1], *N. Salas-Massó*[1]
[1]*Universitat Rovira i Virgili- IISPV,*
Unidad de Microbiología - Departamento de Ciencias Médicas Básicas - Facultad de Medicina y Cien cias de la Salud, Reus, Spain

Backgrounds

The genus *Arcobacter* belongs to the family *Campylobacteraceae* and differs from *Campylobacter* on the basis of the capacity of the species to growth at low temperatures. Since its description in 1991, 24 species had been added to the genus.

Objectives

In the present study we performed a taxonomic study using a polyphasic approach including genome information of two strains (RW43-9 and RW17-10) recovered from secondary treated wastewater.

Methods

The first identification with the *rpoB* gene sequencing, revealed on the basis of the phylogenetic analysis that those strains formed two potentially new linages. A phylogenetic analysis of the 16S rRNA gene and of the concatenated sequences of 5 housekeeping genes (*atpA*, *gyrA*, *gyrB*, *hsp60* and *rpoB*) i.e. MLPA was performed. In addition, genomic DNA was sequenced and used for genomic comparison. The Average Nucleotide Identity (ANI) and the *in silico* DNA-DNA hybridization (*is*DDH) was calculated between the genomes of the two new taxa and their nearest species. Phenotypic characterization of these two strains was also performed.

Conclusions

The differential phenotypic characteristic between the strains RW 43-9 and RW17-10 and its nearest species are the ability to growth in TSA supplemented with 5% sheep blood at 37ºC and 42ºC in anaerobiosis and the inability to growth in minimal medium, respectively. These findings together the MLPA analysis and the values of ANI (<96%) and isDDH (<75%), demonstrate that these strains represent two new species, for which the names *Arcobacter lacus* (type strain RW43-9^T=CECT 8994^T=LMG 29062^T) and *Arcobacter caeni* (type strain RW17-10^T =CECT 9140^T=LMG 29151^T) are proposed.

FEMS7-0585
Taxonomy / Systematics

CREMEIBACTER MARIS GEN. NOV., SP. NOV., FROM COASTAL MEDITERRANEAN SEAWATER

M.J. Pujalte[1], L. Rodrígo-Torres[2], T. Lucena[2], D.R. Arahal[1]
[1]Universitat de Valencia, Microbiología y Ecología, Valencia- Burjassot, Spain
[2]Universitat de Valencia, CECT, Valencia, Spain

Backgrounds

The *Rhodobacteraceae* family, on the *Alphaproteobacteria,* includes more than a hundred genera, most of them isolated from marine environments. Strain CECT 7735^T, isolated from beach seawater (Valencia, Spain) on marine R2A medium, was related by 16S rRNA gene sequence phylogeny to the *Roseobacter* clade of the family. *Phaeobacter gallaeciensis* DSM 26640^T, *Thalassobius abyssi* JAMH043^T and *P. inhibens* DSM 16374^T were the closer relatives, with 97.5 to 97.1% 16S similarity.

Objectives

Phylogenetic, phenotypic, chemotaxonomic and genomic characterization of CECT 7735^T.

Methods

Phenotypic and chemotaxonomic determinations were performed by techniques widely used in taxonomic studies. 16S rRNA gene sequence was compared to those of species with validly published against EzBioCloud database. Illumina Miseq platform was used for genome sequencing. Read analysis, assembly and annotation was done with software included in Galaxy Orione Server and RAST server. Average Nuclotide Indexes (ANI) were calculated with applications in http://jspecies.ribohost.com and OrthoANI software and estimated DNA-DNA hybridization (eDDH) using Genome to Genome Distance Calculator (http://ggdc.dsmz.de).

Conclusions

CECT 7735^T is Gram-negative, chemoorganotrophic, strictly aerobic and slightly halophilic. It requires Na, Mg and Ca ions for growth. Preferred carbon sources are aminoacids. It exhibits 70.3-70.5 ANI and 20.1 to 21.5% eDDH to other *Phaeobacter* species. Phylogenetic analysis with 16S RNA gene sequences does not show a close relationship to *Phaeobacter* or *Pseudophaeobacter* species. Thus, it is considered to represent a novel genus and species, for which the name *Cremeibacter maris* gen. nov., sp. nov. is proposed. The type strain is CECT 7735^T (=LMG 29909^T).

FEMS7-1030
Taxonomy / Systematics

MOLECULAR SYSTEMATICS OF THE GENUS ACIDITHIOBACILLUS: INSIGHTS INTO THE PHYLOGENETIC STRUCTURE AND DIVERSIFICATION OF THE TAXON

R. Quatrini[1], A. Moya-Beltrán[1,2], H. Nuñez[1], P.C. Covarrubias[1], F. Issotta[1], J.P. Cardenas[3], M. Gonzalez[1], J. Atavales[1], L.G. Acuña[1], D.B. Johnson[4]
[1]Fundación Ciencia para la Vida, Microbial Ecophysiology Laboratory, Santiago, Chile
[2]Universidad Andres Bello, Facultad de Ciencias Biologicas, Santiago, Chile
[3]uBiome, San Francisco, USA
[4]Bangor University, College of Natural Sciences, Bangor, United Kingdom

Backgrounds

The acidithiobacilli are sulfur-oxidizing bacteria that thrive in both natural and anthropogenic acidic environments. They contribute to the generation of acid rock drainage in several different geoclimatic contexts, and their properties have long been harnessed for the biotechnological processing of minerals. Presently, the genus is composed of seven validated species, described between 1922 and 2015: *Acidithiobacillus thiooxidans*, *A. ferrooxidans, A. albertensis*, *A. caldus*, *A. ferrivorans*, *A. ferridurans* and *A. ferriphilus*. However, a large number of strains and sequence clones have been obtained over the years, many of which remain unclassified or are miss-assigned, muddling the picture from an evolutionary standpoint.

Objectives

Our goals were to investigate the phylogenetic relationships within this species complex and revise the phylogenetic species boundaries.

Methods

For that purpose we collected sequences affiliated to *Acidithiobacillus* spp. from public and private databases and used three different typing approaches with varying degrees of resolution: 16S rRNA gene-based ribotyping, oligotyping, and multi-locus sequencing analysis.

Conclusions

Results obtained indicate that there is still considerable unexplored diversity within this genus. At least six new lineages, supported by the different methods used herein, were identified. Although the diagnostic characteristics of these subgroups of strains are as yet unresolved, correlations to specific metadata hint to the mechanisms behind econiche-driven divergence of some of the species/phylotypes identified. The emerging phylogenetic structure for the genus outlined in this study will guide isolate selection for future population genomics and evolutionary studies in this important acidophile model.

Acknowledgements: Fondecyt 1140048, Basal CCTE PFB16 and UNAB and Conicyt Scholarship

FEMS7-1803
Taxonomy / Systematics

DEFINING THE SPECIES MICROMONOSPORA SAELICESENSIS UNDER THE FRAMEWORK OF GENOMICS

R. Riesco[1], *C. Prieto*[2], *V. Formamid*[1], *M.E. Trujillo*[1]
[1]*Edificio Departamental- Universidad de Salamanca, Microbiología y Genetica, Salamanca, Spain*
[2]*NUCLEUS- Edificio I+D+i, Servicio de Bioinformática, Salamanca, Spain*

Backgrounds

Current prokaryotic taxonomy uses a polyphasic approach, considering multiple aspects of the microorganism, including phenotypic, genotypic and chemotaxonomic characteristics. However, this approach has serious shortcomings, particularly in the reproducibility of some phenotypic methods. The introduction and improvement of cost-effective whole-genome sequencing methods has provided a new working framework. Unlike DNA-DNA hybridization, the “gold standard” for defining genomic species, genomic data can be stored and made available to the scientific community for subsequent comparisons. In turn, genomic data can also be used to predict phenotypic traits reliably.

Objectives

The aim of the present study was to analyse general overall genome relatedness indices (OGRI) to define the species *Micromonospora saelicesensis* as compared to the polyphasic approach. Genome sequences were also used as a mining source for the prediction of the expected phenotype**.**

Methods

Whole-genome sequences were obtained for sixteen strains identified as *Micromonospora saelicesensis* using 16S rRNA gene, multilocus sequence analyses, DNA-DNA hybridization and metabolic profiles. The data was analyzed using the OGRI methods and compared to previous results. In addition, a series of *in-vitro* phenotypic assays performed at different times were confronted with *in-silico* predictions.

Conclusions

Comparative genomic results showed high variability among the strains studied, indicating that some of them may represent new species. *In-vitro* phenotypic test showed discrepancies among the independent studies, confirming the lack of reproducibility even within the same laboratory. Finally, the use of *in-silico* predictions proved useful for defining a specific phenotype among the strains analyzed.

FEMS7-0593
Taxonomy / Systematics

THALASSOBIUS AUTUMNALIS SP. NOV., A NEW ROSEOBACTER ISOLATED FROM SEAWATER SURROUNDING CULTIVATED OYSTERS

L. Rodrigo Torres[1], *M.J. Pujalte*[1], *T. Lucena*[1], *D. Ruiz*[1]
[1]*Universitat de Valencia, Microbiology and Ecology, Valencia- Burjassot, Spain*

Backgrounds

The genus *Thalassobius* was described by Arahal et al. in 2005 and currently comprises 7 species. This genus belongs to the *Rhodobacteraceae* family, of the *Alpha-proteobacteria,* which includes 128 genera and more than 400 species, most of them from marine environments.

Strains XSM11 and 11SM11 were isolated in November 1989 from seawater (Vinaroz, Spain). The 16S rRNA gene sequence phylogeny placed these isolates within the genus *Thalassobius*, with *T. mediterraneus* CECT 5383^T (99.6% similarity), *T. gelatinovorus* CECT 4357^T (97.6%), and *T. aestuarii* DSM 15283^T (97.4%) as the closest relatives.

Objectives

Phylogenetic, phenotypic, chemotaxonomic and genomic characterization of strains XSM11 and 11SM11.

Methods

Phenotypic and chemotaxonomic determinations were performed by techniques widely used in taxonomic studies. 16S rRNA gene sequence was compared to those of species with validly published against EzBioCloud database. Illumina Miseq platform was used for genome sequencing. Read analysis, assembly and annotation was done with software included in Galaxy Orione Server and RAST server. Average Nuclotide Indexes (ANI) were calculated with applications in http://jspecies.ribohost.com and OrthoANI software and estimated DNA-DNA hybridization (eDDH) using Genome to Genome Distance Calculator (http://ggdc.dsmz.de).

Conclusions

The strains were Gram-negative, non-motile, chemoorganotrophic, aerobic and slightly halophilic, with complex ionic requirements. They exhibited less than 80 % ANIb and less than 25 % eDDH to the types of any other *Thalassobius* species, but 99.9 % and 98.5 %, respectively on these indexes, to each other. On the basis of the results, strains XSM11 and 11SM11 are considered to represent a novel species of the genus *Thalassobius*, for which *Thalassobius autumnalis* sp. nov. is proposed. The type strain is XSM11^T (=CECT 5118^T =LMG 29904^T).

FEMS7-1683
Taxonomy / Systematics

MALDI-TOF MS PROFILES OF REFERENCE STRAINS TO IMPROVE IDENTIFICATION OF DRINKING WATER ASSOCIATED BACTERIA

M.A. Ruvira[1], L. Rodrigo Torres[1], M.C. Macián[1], D. R. Arahal[1], M.J. Pujalte[1], L. Sala-Comorera[2], C. García-Aljaro[2], D. Toribio[2], A. R. Blanch[2], F. Lucena[1], B. Galofré[3], G. Saucedo[3], C. Vilaró[3], S. Fernández[4], D. Baquero[4], R. Aznar[1]
[1]Spanish Type Culture Collection CECT- University of Valencia, Department of Microbiology and Ecology, Paterna Valencia, Spain
[2]Faculty of Biology. University of Barcelona, Department of Genetics- Microbiology and Statistics., Barcelona, Spain
[3]Aigües de Barcelona, Empresa Metropolitana de Gestió del Cicle Integral de l'Aigua- SA, Barcelona, Spain
[4]Cetaqua, Water Technology Center, Barcelona, Spain

Backgrounds

This work is part of an ongoing project, which aims at developing a database of the culturable fraction of bacteria present in drinking water (Drinking Water Library) to be used by drinking water laboratories to improve the efficiency in the management of this resource.

Objectives

To improve the accuracy and robustness of MALDI-TOF MS identification technique of drinking water associated bacteria by widening the available MALDI-TOF MS profiles coverage of reference strains.

Methods

A total of 203 reference strains deposited in CECT were selected according to: source of sample (related to drinking water), culture medium (R2A agar), optimum growth temperature (20-30ºC) and strains belonging to genera that were reported as related to drinking water.

Sample preparation for MALDI-TOF MS was carried out using the ethanol/formic acid extraction protocol recommended by Bruker Daltonics. The spectra measurements were recorded with UltraFleXtrem (Bruker Daltonics) mass spectrometer. The Flexanalysis and Biotyper 3.1 software (Bruker Daltonics) was used for the analysis of the spectra.

A total of 568 (out of 1014) water isolates (bottled mineral water, mineral source water and drinking water utility). remained unidentified after comparison with the current commercial database. After profiles clustering, representative isolates were selected and compared to the enlarged database.

Conclusions

Despite of the 203 new profiles included in the Drinking Water Library only one of the representative isolates could be identified at genus level. Therefore this database still needs to be completed. Further work is ongoing to disclose the identity of the remaining representative isolates that might even represent new species.

FEMS7-2718
Taxonomy / Systematics

PAENIBACILLUS SP. LPB0068T, A NOVEL SPECIES ISOLATED FROM PACIFIC OYSTER

S.K. Shin[1], S. Choi[1], H. Yi[2,3]
[1]Korea University, BK21PLUS Program in Embodiment: Health-Society Interaction. Department of Public Health Sciences. Graduate School, Seoul, Republic of Korea
[2]Korea University, BK21PLUS Program in Embodiment: Health-Society Interaction. Department of Public Health Sciences. Graduate School-, Seoul, Republic of Korea
[3]Korea University, School of Biosystem and Biomedical Science, Seoul, Republic of Korea

Backgrounds

A novel endospore-forming, rod-shaped, Gram-stain-positive bacterial strain, designated LPB0068^T, was isolated from Pacific oyster in Korea.

Objectives

The aim of this study was to investigate the taxonomic position of the new isolate by a polyphasic approach and genome sequence analysis.

Methods

Morphological, cultural, biochemical, physiological, chemotaxonomic tests and phylogenic analysis were performed. Major cellular fatty acids, polar lipids, and menaquinone were determined. The whole genome sequences of strain LPB0068^T and their closely related strains were determined. The genomic relatedness of strain LPB0068^T to other *Paenibacillus* species was calculated by the BLAST-based average nucleotide identity (ANI) score and digital DNA-DNA hybridization (dDDH).

Conclusions

Gene sequence analysis of the 16S rRNA indicated that this isolate is closely related to *Paenibacillus macquariensis* subsp. *macquariensis* DSM 2^T (98.1 %), and *Paenibacillus macquariensis* subsp. *defensor* JCM14954^T (98.0 %). The genome of strain LPB0068^T consists of a circular chromosome with a total length of 4.6 Mb and three circular plasmids of 20, 21, and 87 Kb. The G+C content was 40.0 mol%. ANI and dDDH revealed that strain LPB0068^T is independent from other *Paenibacillus* species. Phenotypic features also distinguished the isolate from related species. On the basis of phenotypic and genotypic results, strain LPB0068^T merits a novel species status of the genus *Paenibacillus*. This study was funded by the Survey of Korean Indigenous Species Program through the National Institute of Biological Resources funded by the Korean Ministry of Environment.

FEMS7-1504
Taxonomy / Systematics

GENOME-BASED INFERENCE OF LATE ACQUISITION OF OXYGENIC PHOTOSYNTHESIS AND AEROBIC RESPIRATION IN THE CYANOBACTERIA

R. Soo[1], J. Hemp[2], D. Parks[1], W. Fischer[2], P. Hugenholtz[1]
[1]Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, Brisbane, Australia
[2]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, USA

Backgrounds

Cyanobacteria are one of the most important microbial groups on Earth with the origin of oxygenic photosynthesis in this phylum leading to the rise of oxygen ~2.3 billion years ago. This event altered the course of evolution by facilitating the development of aerobic respiration and complex multicellular life. However much remains to be learned about cyanobacterial diversity and evolution. For example, the *Melainabacteria,* a non-photosynthetic lineage sister to the photosynthetic Cyanobacteria, was recently described based on genome recovery from metagenomic datasets. Environmental 16S rRNA gene surveys suggest that there are at least three extant classes of Cyanobacteria: *Oxyphotobacteria* (comprising all recognised photosynthetic Cyanobacteria), *Melainabacteria*, and a basal branching group *ML635J-21*, for which genome sequences have yet to be reported.

Objectives

To obtain the first genomic representation of *ML635J-21* and additional *Melainabacteria* genomes, in order to provide insights into the evolution of oxygenic photosynthesis and aerobic respiration in the Cyanobacteria.

Methods

We assembled and binned three draft genomes belonging to class *ML635J-21* from public metagenomic datasets, for which we propose the name *Sericytochromatia.* In addition, we obtained 28 *Melainabacteria* genomes from environmental metagenomes and discovered 10 previously misclassified *Melainabacteria* genomes in public databases. Genomes were assessed for the presence of marker genes of oxygenic photosynthesis and aerobic respiration, and phylogenetic trees were inferred from identified Complex III and IV genes.

Conclusions

All members of the *Melainabacteria* and *Sericytochromatia* lack photosynthetic machinery indicating that phototrophy was not an ancestral feature of the Cyanobacteria, and that *Oxyphotobacteria* acquired the genes for photosynthesis relatively late in cyanobacterial evolution. Independent acquisition of aerobic respiratory complexes in the three classes supports the inference of late acquisition of oxygenic photosynthesis.

FEMS7-0108
Taxonomy / Systematics

INTRODUCING THE SPOROBIOME: AN UNEXPLORED NEXUS OF RESISTOME AND NOVEL HUMAN PATHOGENS

G. Tetz[1]
[1]*Human Microbiology Institute, Human Microbiology, New York, USA*

Backgrounds

Microbiomes can be categorized based on common features, allowing the performance of deeper analyses. For example subdivisions—virome, fungal biome, resistome—are based on the totality of all genes of a specific microbial population. Spore-forming bacteria, the diversity of which is considered to be underestimated, possess unique features that differentiate them from other microorganisms. Bacterial endospores render the resistance to unfavorable environmental conditions. This provides challenges in treating such infections: higher rates of transmission; persistence ; high rates of relapses ; presence of sporulation-related virulence factors; protection from the immune response and antibacterials. Here, we suggest that studying the totality of genes of spore-forming bacteria, which we propose to name sporobiome, will facilitate our understanding of their role in health and diseases.

Objectives

Pure cultures of 30 different species of spore-forming bacteria including 12 previously unknown species, were isolated. The complete genome sequences of the previously unknown species were deposited in GenBank. Analyzing the sporobiome of these bacteria revealed multiple genes encoding virulence factors and antibiotic resistance, including virulence factors associated solely with spore-forming bacteria. Expression of antibiotic-resistance genes was studied.

Methods

Previously unculturable, aerobic, spore-forming bacteria were isolated from the microbiota of patients with lung infections and cancer. We identified bacteria by using by using a novel workflow that is combination of microbiological, biochemical (Vitek 2, MALDI-TOF), and genetic (Illumina HiSeq 2500) analyses. De novo assembly was performed using SPAdes genome assembler software. Antibiotic susceptibility was determined via the disc diffusion method and Vitek2.

Conclusions

Our approach allows to evaluate previously unknown spore-forming bacteria within human microbiome. We suggest that further study of the sporobiome will facilitate understanding its unique role in disease, the spread of genetic information among different ecological niches, and the maintenance and spread of antibiotic-resistance genes.

FEMS7-1145
Taxonomy / Systematics

PLEOMORPHOCHAETA CAUDATA GEN. NOV., SP. NOV. AND PLEOMORPHOCHAETA OLEARIA SP. NOV., TWO BIOSURFACTANT-PRODUCING STRAINS FROM AN OFFSHORE OIL FIELD

B. Arroua[1], L. Urios[1], M. Ranchou-Peyruse[1], M. Magot[1], P. Bouriat[2], A. Ranchou-Peyruse[1], R. Grimaud[1]
[1]IPREM UMR CNRS 5254 - Université de Pau et des Pays de l'Adour, Equipe Environnement et Microbiologie, 64013 Pau Cedex, France
[2]IPRA - FR 2952 - Université de Pau et des Pays de l'Adour, LFC-R, 64013 Pau Cedex, France

Backgrounds

Petroleum reservoirs harbour microbial communities with a wide range of activities. These microorganisms can impact oil quality and recovery through detrimental effects such as corrosion of pipeline and hydrocarbon biodegradation and/or beneficial effects like microbial enhanced oil recovery (MEOR). MEOR consists in injecting allochthonous or stimulating indigenous bacteria that increase oil recovery by altering oil/water/rock interface properties or rock permeability.

Objectives

Activities linked to MEOR were explored for two strains, named SEBR 4223 and SEBR 4209, that were isolated from produced water of an offshore oil field.

Methods

Biosurfactant production was tested by measurement of interfacial activity of the culture supernatant with cyclohexane using a pendant drop tensiometer. The taxonomic characterization of strains SEBR 4223 and SEBR 4209 has been achieved by following a polyphasic approach.

Conclusions

Both strains were able to lower the interfacial tension, suggesting the production of a biosurfactant. Results obtained in this study suggested that these strains represent two novel species of a new genus belonging to the *Spirochaetaceae*. Considering the important pleomorphism of the cells, the names *Pleomorphochaeta*, for this new genus, *Pleomorphochaeta caudata* and *Pleomorphochaeta olearia*, for strains SEBR 4223 and SEBR 4209 respectively, are proposed.

FEMS7-2256
Taxonomy / Systematics

CULTURE-DEPENDENT STUDY OF ARCHAEA AND BACTERIA FROM SALTERNS AND SALINE SOILS WITH DIFFERENT SALINITY RANGES

A. Durán-Viseras[1], M.J. León[1], C. Sánchez-Porro[1], A. Ventosa[1]
[1]University of Seville, Dept Microbiology and Parasitology, Sevilla, Spain

Backgrounds

Recent metagenomic studies on ponds of salterns with different salinities (from ca. 10 % to salt saturation) have shown that the microbial diversity is strongly influenced by the salinity and permitted to determine the major microbial groups that inhabit ponds with increasing salinities as well as their activities. Most studies have been focused on the higher salinity ponds (designated as crystallizers), with haloarchaea, nanohaloarchaea and some Bacteroidetes as dominating populations. However, the intermediate salinity saltern ponds have not been studied in detail

Objectives

Metagenomic data indicate that a large percentage of the prokaryotes in intermediate to high salinity ponds have not yet been isolated in pure culture. The aim of this study is the isolation and characterization of the archaea and bacteria which may be the dominant population on these saline habitats.

Methods

Clasical and new methodologies have been used for the isolation of halophilic archaea and bacteria under different growth conditions. They have been preliminary identified by partial 16S rRNA gene sequencing analyses.

Conclusions

We have isolated a large collection of strains which belong to the haloarchaea and halophilic bacteria (moderately and extremely). Most of these strains belong to previously reported taxa, as inhabitants of hypersaline environments even though they a large proportion are members of genera that are most frequently isolated in laboratory media but are not constituting a large percentage of the microbial population, as reported by molecular approaches. On the other hand, we have isolated some new strains which may represent new taxa and their features are under investigation.

FEMS7-1520
Taxonomy / Systematics

PROPOSAL TO RECLASSIFY THE CLASS EPSILONPROTEOBACTERIA AS A NEW PHYLUM; EPSILONBACTERAEOTA

D. Waite[1], I. Vanwonterghem[1], C. Rinke[1], D. Parks[1], Y. Zhang[2], K. Takai[3], S. Sievert[4], J. Simon[5], B. Campbell[6], T. Hanson[7], T. Woyke[8], M. Klotz[9], P. Hugenholtz[1]
[1]Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, Brisbane, Australia
[2]University of Rhode Island, Department of Cell and Molecular Biology, Kingston, USA
[3]Japan Agency for Marine-Earth Science and Technology, Institute of Biogeosciences, Kanagawa, Japan
[4]Woods Hole Oceanographic Institution, Biology Department, Woods Hole, USA
[5]Technische Universität Darmstadt,
Microbial Energy Conversion and Biotechnology- Department of Biology, Darmstadt, Germany
[6]Clemson University, Department of Biological Sciences, Clemson, USA
[7]University of Delaware, School of Marine Science and Policy, Newark, USA
[8]DOE Joint Genome Institute, DOE Joint Genome Institute, Walnut Creek, USA
[9]The City University of New York,
Department of Biology and School of Earth and Environmental Sciences, New York, USA

Backgrounds

The *Epsilonproteobacteria* is the fifth validly described class of the phylum Proteobacteria, known primarily for clinical relevance and chemolithoautotrophy in various terrestrial and marine environments, including deep-sea hydrothermal vents. However, the phylogenetic placement of this class has become less certain as 16S rRNA gene repositories expand and additional molecular markers are considered. For example, many concatenated marker gene analyses have failed to recover the *Epsilonproteobacteria* as monophyletic with other classes of Proteobacteria.

Objectives

We sought to evaluate the placement of *Epsilonproteobacteria* within the bacterial tree of life, and to assess the suitability of their continued inclusion within the Proteobacteria using comparative genomics.

Methods

A data set of 22,773 bacterial genomes (including 653 *Epsilonproteobacteria*) was obtained and analysed using both ribosomal RNA and 120 single copy protein markers. Evidence for the inclusion of *Epsilonproteobacteria* within the phylum Proteobacteria was assessed based on single- and concatenated-gene trees using a variety of tree building methods. Sequential jackknifing of outgroup phyla from the data set was performed to test the robustness of phylogenetic affiliations under differing combinations of bacterial genomes.

Conclusions

Based on the assessment of almost 300 phylogenetic tree topologies, we conclude that the continued inclusion of *Epsilonproteobacteria* within the Proteobacteria is not warranted, and that this group should be reassigned to the novel phylum Epsilonbacteraeota (*phyl. nov.*). We further recommend the reclassification of the order *Desulfurellales* (*Deltaproteobacteria*) to a novel class within this phylum. We also recommend a number of subordinate changes at the family level, to ensure consistency with the genome-based phylogeny.

FEMS7-2275
Taxonomy / Systematics

GENOME CHARACTERIZATION OF STRAIN KB1, A NOVEL MEMBER OF THE GENUS BLAUTIA ISOLATED FROM A HUMAN FECAL SAMPLE

S.J. Yang[1], N.R. Shin[2], W. Kang[2], B. Jin-Woo[2], K. Byung-Yong[1]
[1]ChunLab- Inc., Bioinformatics Reserach Institute, Seoul, Republic of Korea
[2]Kyung Hee University,
Department of Life and Nanopharmaceutical Sciences and Department of Biology, Seoul, Republic of Korea

Backgrounds

The genus of *Blautia* is a member of the family *Lachnospiraceae* and now contains eleven validly published species. Most of species in this genus are Gram positive, coccoid, and strictly anaerobic bacteria found in human fecal samples. According to recent studies, *Blautia* is a one of most abundant group in gastrointestinal(GI) and found that level of this group was a strong indicator of a gut health.

Objectives

In this study, we present the genome characters of strain KB1, a novel bacterium of the *Blautia* genus.

Methods

The strain KB1, belonged to the *Blautia* genus was isolated from a human fecal sample in an anaerobic condition using brain-heart infusion(BHI) agar. The genome was sequenced on the MiSeq platform of Illumina using a paired-end library.

Conclusions

Phylogenetic analysis based on 16S rRNA gene and whole genome sequences by average nucleotide identity (ANI) showed that strain KB1 is closely related to *Blautia* species. The genome was assembled to 49 contigs and total length of the contigs was 6,044,239 nucleotides with a G+C content of 46.3%. The genome harbors 14 rRNA genes, 64 tRNA genes, 5,152 CDS. The genome was predicted to have complete gene sets for glycolysis, citric acid cycle, pentose phosphate pathway and PRPP biosynthesis. Based on genome characteristics presented in this study, we propose to the strain KB1 as represents a novel species within the genus of *Blautia*.

Acknowledgement [This work was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT & Future Planning(2015M3C9A2054320)]

FEMS7-3062
Taxonomy / Systematics

PROTEOMIC APPROACH FOR THE IDENTIFICATION OF BACILLUS SPECIES USING MATRIX-ASSISTED LASER DESORPTION/IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY

W.S. Yu[1], H.M. Chenong[1], H.N. Do[1], K.J. Hwang[1]
[1]Korea National Institute of Health,
Pathogen Resource TF- Center for Infectious Diseases- Korea National Institute of Health- Korea Center for Disease Control and Prevention, Cheongju, Republic of Korea

Backgrounds

The genus *Bacillus*, consisting of phylogenetically and phenotypically diverse species, represents rod-shaped, endospore-forming bacteria, including species of medical importance. The application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has greatly facilitated bacterial identification.

Objectives

In this study, we compared various methods of taxonomic identification of *Bacillus* strains:the VITEK 2 (bio-Mérieux, France), 16S rRNA gene sequencing and MALDI-TOF MS. We also supplemented the main spectrum profiles (MSPs) of the reference strains and clinical isolates with MALDI Database (Bruker Daltoniks, Germany), thus increasing rates of identification when using MALDI-TOF MS.

Methods

Thirty *Bacillus* strains were obtained from the National Culture Collection for Pathogens in Korea and were identified using the VITEK 2, 16S rRNA gene sequencing and MALDI-TOF MS. The pathogenicity of the *Bacillus* strains was confirmed through the identification of virulence genes by multiplex PCR. The dendogram of MSPs was compared with the phylogenetic tree constructed based on the 16S rRNA gene sequences and rep-PCR fingerprinting.
The rates of species-level identification were 40%, 80%, and 76.3% using the VITEK 2, 16S rRNA gene sequencing and MALDI-TOF MS, respectively. Thirty new entries of MSPs were manually entered into our MALDI database, which included the entire Bruker MALDI database. The results of phylogenetic analysis showed that MALDI-TOF MS is an effective tool for identification of *Bacillus* strains.

Conclusions

Bacillus strains were more efficiently identified using MALDI-TOF MS and 16S rRNA gene sequencing than the VITEK 2. Continual addition of MSPs to a proteome-based database can result in increased rates of identification using MALDI-TOF MS.

FEMS7-0027
Virology

MOLECULAR DETECTION OF BIODIVERSITY OF TOMATO VIRUSES IN EGYPT AND PHYLOGENY ANALYSES OF SOME DETECTED ISOLATES

E. Abdel Aleem[1], C. Ratti[2], M. Rabie[1], F.A. Fattouh[1]
[1]Botany and MicrobiologyDepartment, Faculty of Science, Alexandria University, Alexandria, Egypt
[2]DipSA – Patologia Vegetale, Università di Bologna, Viale G. Fanin, 40 - 40127 Bologna, Italy

Backgrounds

Lycopersicon esculentum (tomato) is recognized as the second significant vegetable crop in the world. Egypt is the fifth largest producer of tomatoes in the world while over 9 million tons of tomatoes are produced annually. Tomatoes, a major export crop, contribute significantly to Egyptian economy.

Objectives

Tomato is subject to infection by a number of plant viruses that affect its yield and quality.

Methods

Recent surveys for the detection of tomato viruses by RT-PCR using synthetic specific oligonucleotide primers have allowed the identification of three major viruses infecting tomato. *Tomato yellow leaf curl virus* (TYLCV), *Cucumber mosaic virus* (CMV) and *Tomato mosaic virus* (ToMV) were detected in major tomato growing areas surveyed. Several isolates of TYLCV, CMV and ToMV were selected for further molecular characterization through sequencing of key genome parts of each. Full length sequencing was performed for one isolate of each of the three viruses. Phylogenetic analyses were performed which allowed the identification of closely related isolates in other parts of the world.

Conclusions

To our knowledge, this is the first characterization of CMV isolates from subgroup IB and the first report of ToMV in Egypt. Our results give a direct indication of the necessity to focus efforts on viral disease surveillance and to face the challenges of international trade in agriculture products for the proper management of those diseases.

FEMS7-3004
Virology

HEALING THE YEAST FROM L-A VIRUS(ES)

L. Aitmanaitė[1], A. Konovalovas[1], E. Servienė[2,3], S. Serva[1,3]
[1]Vilnius University, Department of Biochemistry and Molecular Biology, Vilnius, Lithuania
[2]Nature Research Centre, Laboratory of Genetics, Vilnius, Lithuania
[3]Vilnius Gediminas Technical University, Department of Chemistry and Bioengineering, Vilnius, Lithuania

Backgrounds

Yeast L-A virus is a member of Totiviridae family, representing the group of viruses widespread among fungi and protozoa. Its single segment dsRNA genome encodes a major coat protein Gag and a Gag-Pol fusion protein, which functions as RNA-dependent RNA polymerase. Some wild yeast strains in addition to widespread L-A carry a satellite called M dsRNA. Satellite dsRNA genome solely encodes a secreted protein toxin, thus providing coding cell with survival advantage. L-A spreads by direct cytoplasm contacts occurring during cell division and cell-cell mating, while extracellular stage of these viruses remains unknown.

Objectives

Global gene expression analysis of yeasts focusing on the impact of L-A dsRNA should reveal molecular mechanisms involved in the maintenance of virus. In order to obtain relevant RNA-seq results, an efficient procedure for eliminating of L-A virus from yeast is required. Until now, three main approaches were used: treatment by heat, cycloheximide, and 5-fluorouracil. All listed treatments are cytotoxic for yeast and therefore could result in false positive hits in -omics experiments.

Methods

Our group constructed expression vectors of different L-A virus Gag proteins. The impact of Gag protein expression on native L-A virus was examined by nucleic acid electrophoresis, RT-PCR and killer phenotype assay.

Conclusions

Overexpression of L-A capsid protein Gag results in complete L-A virus elimination from yeast. L-A elimination occurs not only in case of overexpression of genuine capsid protein; capsid proteins from some of the different type L-A viruses are competent for exclusion, also. The obtained data highlight the evolutionary relationship between various types of L-A viruses, sharing molecular mechanisms for virus maintenance in yeast.

FEMS7-1510
Virology

BIOINFORMATIC ANALYSIS OF L1, E6 AND E7 SEQUENCES FROM HUMAN PAPILOMA VIRUSES OF HIGHEST AND LOW RISK MOST FREQUENTLY IN LATIN AMERICA

A. Angulo[1], M.C. Yépez[1], M. Guerrero[1]
[1]University of Nariño, Centro de Estudios en Salud - CESUN, Pasto, Colombia

Backgrounds

Intratype HPV diversity in virus molecular variants leading alterations to changes in the residues of its functional domains related to the persistence of infection and the progression of cervical cancer precursor lesions (CCU) has been revealed. Variations in virus nucleotide sequences can be used as a tool in epidemiological studies to identify biomarkers, and clarify the virulence, expression and transformation of host cells, as well as taxonomic and evolutionary HPV studies.

Objectives

To analyze nucleotide sequences of the L1, E6 and E7 genes, to recognize the phylogenetic relationships, variants of genotypes and geographic distribution of the most frequent high and low risk HPV from Latin America.

Methods

Phylogenetic approximations from L1 gene sequences under the maximum likelihood criterion using the MEGA v.6.0.5 program were made. Variability analysis of E6 and E7 genes was performed with software BioEdit V. 7.2.5, which allowed the identification of punctual mutations for specific variants of each genotype of HPV, which we later projected on maps to establish their distribution with ArcGis v. 10.2.

Conclusions

770 sequences from 13 countries, mainly Costa Rica, Mexico and Brazil, were obtained for HPV types 6, 11, 16, 18, 31, 33, 35, 45, 52, 58 and 97. 24 new mutations for specific HPV types, except to HPV 6, 16, 31 and 35. This results allowed to identify point mutations that positively could correlated with the persistence of the virus and the NIC grade II and III, and give to these genotypes evolutionary advantages and adaptive mechanisms.

FEMS7-1661
Virology

DRAFT GENOME SEQUENCE OF BACTERIOPHAGE BK30P, LYTIC PHAGE THAT INFECTS MACROMONAS

K. Baek[1], *A. Choi*[1]
[1]*Nakdonggang National Institute of Biological Resources, Freshwater Bioresources Research, Sangju-si, Republic of Korea*

Backgrounds

Bacteriophages lyse specific bacterial hosts, controlling bacterial abundance and diversity and influencing biogeochemical cycles, which makes the study of viruses ecologically important.

Objectives

we present the first draft genome sequence of this strain.

Methods

Genomic DNA was extracted from strain BK30P using a DNeasy blood and tissue kit. Genome sequencing was performed using the Illumina sequencing technology with library constructed. The bacterial genome was assembled *de novo* into one contig. The data was then submitted to the Rapid Annotation using Subsystem Technology (RAST) server and the National Center for Biotechnology Information genome sequence database. Potential coding sequences were searched for using the Basic Local Alignment Search Tool (BLAST) against the Pfam and COG databases.

Conclusions

Bacteriophage BK30P is a lytic bacteriophage that infects the geuns macromonas strain BK30, a Freshwater bacterium affiliated with Burkholderiales. Both the bacteriophage and the host bacterial strain were isolated from surface freshawater samples collected off the Nakdong river of Korea. The phage particle has an icosahedral capsid with a diameter of ~60 nm and a long tail of ~82 nm in length; these characteristics constitute the distinctive morphology of the myoviridae family. The complete genome sequence of phage BK30P is 43,064 bp long with 58.6% G+C content. This complete genome sequence is the first report of a lytic phage that infects macromonasa, for which the name “macromonasphage” is proposed.

FEMS7-1575
Virology

ISOLATION AND CHARACTERIZATION OF A NEW TEMPERATE BACTERIOPHAGE OF VIBRIO ANGUILLARUM

O. Flores[1], K. Paez[1], D. Castillo[2], M. León[1], R. Bastías[1]
[1]Pontificia Universidad Católica de Valparaíso, Instituto de Biología, Valparaíso, Chile
[2]University of Copenhagen, Marine Biological Section, Copenhagen, Denmark

Backgrounds

Vibrio anguillarum is a recurrent pathogen in Aquaculture industry. In marine environments its abundance, diversity and evolution can be influenced by lytic and temperate bacteriophages.

Objectives

The aim of this work was to characterize the new temperate bacteriophage Valp1 of *V. anguillarum* that was isolated from coastal waters in Valparaíso, Chile.

Methods

The phage was characterized by TEM, genome analysis and biological properties, while the lysogenized strain was compared with the *wt* strain in their growth and biochemical profile using the BIOLOG system.

Conclusions

The bacteriophage Valp1 presents an icosahedral head of 60 nm and a tail of 90 nm. A preliminary analysis of its 43 kb genome revealed the presence of 36 ORF and confirmed its temperate nature. The phage was not detected in other *V. anguillarum* strains genomes, but 72% of the ORF presented partial identity with prophage proteins found in *V. cyclitrophicus*. The growth of the *wt* host strain decreases in presence of the bacteriophage (MOI=1), while the growth of the lysogenized strain is slightly lower comparing to the *wt*, but was not affected by the addition of Valp1. The lysogenic strain did not show any alterations in biofilm formation or motility; however presents alterations in 11.7% of the biochemical tests comparing to the *wt* strain. These results suggest that the bacteriophage Valp1 can affect the metabolism of its host. This work is the first step to understand how the phage Valp1 can be influencing the fitness, evolution or even the virulence of *V. anguillarum* in the environment.
Fondecyt 11140412

FEMS7-2742
Virology

SAT, A NOVEL PARVOVIRAL PROTEIN INDUCING CELL LYSIS

C. Bretscher[1], *J. Nüesch*[1]
[1]*German Cancer Research Center, Tumor Virology, Heidelberg, Germany*

Backgrounds

MVMp and H1 are rodent parvoviruses – small, non-enveloped icosahedral particles with a 5.1 kb single-stranded DNA as a genome. Due to their striking oncotropism and oncolytic activities some of these agents are considered as excellent candidates for a virotherapy of cancer. This, together with a strong safety profile led to first phase I/IIa clinical trials targeting glioblastoma multiforma and pancreatic ductal adeno carcinoma.

Objectives

The main parvoviral oncotoxic protein is thought to be the large non-structural protein NS1. However, additional viral factors appear to be required to efficiently permeabilize the plasma membrane and to induce cell lysis. Recently, a short conserved hydrophobic polypeptide termed SAT of presently unknown function was identified in the related porcine parvovirus. SAT contains a transmembrane domain and is found to localize to membranous compartments such as the endoplasmatic reticulum, the Golgi and the plasma membrane. These findings led us to hypothesize that SAT might act as a viroporin, a family of viral proteins that are capable of permeabilizing membranous structures through multimerization within the target compartment.

Methods

In fact, after transfection we could show in an immunoprecipitation, in a proximity ligation assay and in a cell-lysis assay that SAT homo-oligomerizes and in consequence permeabilizes the plasma membrane. Current investigations are focused to determine the oligomeric state of SAT and to determine the impact of distinct motifs in the polypeptide being involved in the lytic process.

Conclusions

Our recent findings strongly support our hypothesis of SAT being involved in pore-formations and release of progeny particles from infected cells.

FEMS7-1702
Virology

VIRUCIDAL ACTIVITY OF GAMMA RADIATION ON ENTERIC VIRUSES

A. Pimenta[1], D. Guerreiro[1], J. Madureira[1], F. Margaça[1], S. Cabo Verde[1]
[1]Instituto Superior Técnico, Centro de Ciências e Tecnologias Nucleares - C2TN, Bobadela LRS, Portugal

Backgrounds

Enteric viruses, such as norovirus (NoV) and adenovirus (AdV), are a major cause of water and foodborne diseases. These viruses are environmentally stable and resistant to conventional treatments and common disinfectants. Among non-thermal treatment methods, ionizing radiation is recognized as a useful and effective means of disinfection.

Objectives

The goal of this study was to investigate the inactivation by gamma irradiation of murine norovirus type 1 (MNV-1), as a NoV surrogate, and human adenovirus type 5 (AdV-5) in six different aqueous substrates and two types of fresh berry fruits.

Methods

Phosphate Buffer Saline, demineralized water, tap water, Fetal Bovine Serum solutions as well as fresh strawberries and raspberries, were inoculated either individually with MNV-1 and AdV-5 or with a viral pool of both viruses. The spiked samples were irradiated in a Co-60 chamber at doses of 1 kGy up to 11 kGy. The infectivity of viral particles of MNV-1 and AdV-5 was tested by plaque assay using Raw 264.7 and A549 cells, respectively. D_{10} values and virucidal efficiency of gamma irradiation were estimated for each virus and substrate.

Conclusions

On wastewater suspensions it was achieved a reduction on MNV and AdV titers of 3 log PFU/ml after irradiation at 5 kGy. For spiked fresh berries irradiated at 4 kGy it was obtained a reduction on virus titers of 2 log PFU/g. But, non-linear inactivation survival curves were obtained for MNV and AdV in fresh fruits, leading to the detection of infective viral particles at a dose of 11 kGy.
The irradiation treatment indicated virucidal activity for the enteric viruses, although the viral inactivation by gamma radiation was found to be dependant on the substrate where the viruses are present. The irradiation technology can be an effective virus mitigation tool to treat polluted waters, which are a major vehicle of contamination for fresh produce.

FEMS7-0700
Virology

IDENTIFICATION OF VIRUSES INFECTING SWEET POTATOES IN KOREA USING NEXT-GENERATION SEQUENCING

Y. Jo[1], H. Choi[1], W.K. Cho[1]
[1]Seoul National University, Department of Agricultural Biotechnology, Seoul, Republic of Korea

Backgrounds

Sweet potato [*Ipomoea batatas* (L.) Lam] is one of the most important food crops and is cultivated worldwide. Although sweet potatoes grow from seeds, most are vegetatively propagated by planting rooted sprouts or slips. Therefore, many sweet potatoes are infected by various viruses, reducing production.

Objectives

In June 2016, we collected several sweet potato samples from six different regions in Korea for the identification of viruses infecting sweet potatoes.

Methods

Pooled samples were subjected to total RNA extraction followed by library preparation. Six different libraries were paired-end sequenced by HiSeq 2000. Sequenced raw data were used for *de novo* transcriptome assembly using Trinity. Assembled contigs were subjected to BLAST search against a viral reference database.

Conclusions

We identified seven known viruses infecting sweet potatoes. Of the six regions, only in the library from Yeongam were none of viruses identified, while the libraries from Iksan and Yeoju each contained a large number of viral reads associated with at least five viruses. Of identified viruses, *Sweet potato virus C* and *Sweet potato feathery mottle virus* were abundantly present in five regions. In many cases, sweet potatoes were co-infected by several viruses. The virus infection rates and the kinds of viruses infecting sweet potatoes were varied by region. This is the first report of viruses infecting sweet potatoes in Korea using next-generation sequencing. Our results will be useful for virus diagnosis and the generation of virus-free sweet potato plants.

FEMS7-0730
Virology

IDENTIFICATION OF VIRUSES INFECTING BARLEY IN KOREA USING NEXT-GENERATION SEQUENCING

H. Choi[1], Y. Cho[1], W.K. Cho[1]
[1]Seoul National Univesity, Department of Agricultural Biotechnology, SEOUL, Republic of Korea

Backgrounds

Barley (*Hordeum vulgare*) is an annual cereal grain belonging to the family *Poaceae* that has been cultivated for more than 7000 years. Although barley is tolerant to abiotic stresses such as cold, drought, and salinity, many kinds of pathogens, including viruses, cause reduction of barley production.

Objectives

In order to examine viruses infecting barley in Korea, we harvested 110 barley plants showing viral disease symptoms from 17 different regions in Korea.

Methods

We pooled samples according to six geographical regions and generated six different libraries for RNA sequencing using next-generation sequencing. Raw data sequenced by HiSeq 2000 were *de novo* assembled using Trinity. The assembled contigs were subjected to BLAST search against a viral genome database.

Conclusions

We identified eight different viruses infecting barley. Of these, *Barley yellow mosaic virus* (BaYMV in the genus *Bymovirus*) was dominant, followed by *Barley mild mosaic virus* (BaMMV in the genus *Bymovirus*) and *Barley yellow dwarf virus* (BYDV in the genus *Luteovirus*). Furthermore, the barley viromes derived from different regions showed region-specific viral communities. In addition, we obtained five nearly complete genomes for BaYMV and two for BaMMV. Moreover, we calculated viral copy number and single nucleotide variations (SNVs) for assembled viral genomes. The number of identified SNVs for each virus was dependent on the cultivated regions. Taken together, this is the first comprehensive study of barley viromes in Korea.

FEMS7-2267
Virology

SINGLE VIRUS GENOMICS, A PROMISING METHODOLOGY TO STUDY THE UNCULTURED HUMAN VIROME.

M.J. de la Cruz Peña[1], F. Martínez Hernández[1], M. Lluesma Gomez[1], M. Garcia[1], O. Fornás[2], L. Díaz[3], M.A. Muñoz Fernández[3], F. Marco[4]
[1]Universidad de Alicante, Department of Physiology- Genetics and Microbiology, Alicante, Spain
[2]Barcelona Biomedical Research Park-Centre for Genomic Regulation, Flow Citometry Unit, Barcelona, Spain
[3]Hospital General Universitario Gregorio Marañón, Department of Immunology, Madrid, Spain
[4]Hospital General Universitario de Alicante, Department of Immunology, Alicante, Spain

Backgrounds

Viruses play significant and different roles in the biosphere and are very abundant in all environments. Our research focused on the uncultured human salivary viruses. Although several surveys have studied the natural viral assemblages in human saliva (Willner et al., 2011; Abeles et al., 2014; Pride et al., 2012), very little is known about differences in viral composition between healthy and immunoglobulin A (IgA), M (IgM) and G (IgG) immunodeficiency people. For instance, IgA acts as an important first line of defense against microbes in the mucous membranes.

Objectives

In the present study we combine Metaviromics and Single Virus Genomics to unveil the genetic information of ecologically predominant viruses present in these contrasting biological conditions of the human mucus.

Methods

Single Virus Genomics allows to obtain the genetic information directly from one virus at a time without the need of culturing the host. First, single viral particles are directly separated by flow cytometry from the saliva by fluorescence activated viral sorting. Then, the genetic material of single sorted viruses was whole genome amplified by multiple displacement amplification and finally sequenced. In parallel, metaviromes from same sample were sequenced and used for a cross genomic comparison with single virus genome datasets

Conclusions

Albeit we are at the beginning of our research, so far, we unveiled the genome of three highly abundant uncultured *Streptococcus* and *Moraxella* phages. This suggests that this promising methodology will open new ways to contrast hypothesis in the human microbiome.

FEMS7-3064
Virology

IMPACT OF GLOSSINA PALLIDIPES SALIVARY GLAND HYPERTROPHY VIRUS ON A HETEROLOGOUS HOST, G. F. FUSCIPES

G. Demirbas Uzel[1,2], A.G. Parker[1], M.J.B. Vreysen[1], R.L. Mach[2], A.M.M. Abd-Alla[1]
[1]International Atomic Energy Agency,
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria
[2]Vienna University of Technology,
Institute of Chemical- Environmental and Biological Engineering- Research Area Biochemical Technol ogy, Vienna, Austria

Backgrounds

Tsetse flies (Diptera: Glossinidae) are the vector of African trypanosomosis, the cause of sleeping sickness in humans and nagana in animals. The development of resistance by trypanosomes to the available drugs makes vector control the most effective approach for sustainable management of nagana. The sterile insect technique (SIT) is an effective method to suppress or eradicate tsetse flies in the frame of an area-wide pest management programme. SIT includes the mass production of the target insect and release of sterile males in the targeted area to compete with wild males in mating with wild females.

Objectives

Tsetse mass rearing is a challenge for SIT application and an understanding of the reproductive biology and associated pathogens is essential to improve mass rearing. *Glossina fuscipes fuscipes* is one of the most important vectors of sleeping sickness in central Africa. GpSGHV affects the fecundity, productivity and therefore performance and sustainability of *G. pallidipes* colonies.

Methods

In this study, we evaluated the impact of GpSGHV on the performance of a *G. f. fuscipes* colony including productivity, mortality, survival, flight ability and insemination rates.

Conclusions

The results indicate that GpSGHV infection has a significant impact on mortality, productivity, survival and mating ability but not on adult flight ability or insemination rates in *G. f. fuscipes*. These results explore the important role of GpSGHV in the sustainability of *G. f. fuscipes* colonies and the need to implement measures to avoid virus infection to ensure the successful establishment of mass rearing of this species for SIT programmes.

FEMS7-0365
Virology

VIRAL TAGGING: IDENTIFYING BACTERIA PREFERENTIALLY INFECTED BY BACTERIOPHAGES

M. Dzunkova[1], *C. Rinke*[1], *J. Daly*[1], *L. Deng*[2], *P. Hugenholtz*[1]
[1]*University of Queensland, Australian Centre for Ecogenomics, Brisbane, Australia*
[2]*Helmholtz Zentrum München, Institute of Virology, Neuherberg, Germany*

Backgrounds

The recently developed viral tagging method allows the host range of viruses to be determined without the need for plaque formation tests. Fluorescently stained bacteriophages from an environmental sample are mixed with a non-stained bacterial host. Bacterial cells infected by phages are collected by the fluorescence activated cell sorting (FACS). The DNA of the infecting phages is then sequenced and analyzed.

Objectives

To date, the viral tagging method has only been applied to bacterial isolates. Our goal was to extend the method to uncultured bacterial species of the human gut microbiome, and to determine the network of bacterial-viral interactions in that ecosystem.

Methods

Viral particles isolated from feces of a healthy volunteer were fluorescently stained and mixed with bacterial cells recovered from the same sample. FACS was used for collection of 100,000 bacterial cells tagged with stained viruses in three replicate sorts, along with the non-tagged cells as controls. The attachment of bacteriophages to the bacterial cell surface was confirmed by electron microscopy. DNA from the collected cells was shotgun sequenced and the bacterial species identified using marker genes.

Conclusions

A subset of the bacterial species in the volunteer's gut microbiome were found to be enriched in the viral-tagged populations. Specifically, *Streptococcus, Clostridium, Blautia, Dorea* and *Ruminococcus* were more abundant in the viral-tagged fraction, while *Bacteroides, Roseburia* and *Sutterella* were under-represented relative to non-tagged controls. Our results suggest that some gut species are more prone to the attack by native bacteriophages. Future investigations will resolve the host-viral interaction network in this ecosystem.

FEMS7-2810
Virology

INACTIVATED NERVOUS NECROSIS VIRUS (NNV) VACCINE ELICITS ANTIVIRAL ACTIVITY AND PROTECTION IN THE TELEOST EUROPEAN SEA BASS (DICENTRARCHUS LABRAX)

E. Chaves-Pozo[1], Y. Valero[1], M. Djamal[2], D. Ceballos-Francisco[3], M.A. Esteban[3], A. Cuesta[3]
[1]Insituto Español de Oceanografía, Mazarrón, Mazarrón, Spain
[2]Université de Blida, 2Institut des Sciences Vétérinaires, Blida, Algeria
[3]University of Murcia, Cell Biology & Histology, Murcia, Spain

Backgrounds

Nodavirus (NNV; *Nodaviridae* family, *Betanodavirus*genus), is one of the most threatening virus for teleost fish and causes the viral encephalopathy and retinopathy (VER) diseasethat alters brain and retina structure and function. Amongmarine fish, the European sea bass (*Dicentrarchus labrax*), a very relevantspecies in Mediterranean aquaculture, is one of the mostsusceptible species, being larvae and juvenile stages those sufferinghighest mortalities. To date, no effective vaccines are available.

Objectives

Our aim was to generate an inactivated NNV vaccine for sea bass and evaluate the immunity and protection.

Methods

Sea bass specimens of 10 g were intraperitoneally injected with UV-inactivated NNV and after 1 month infected with a lethal dose of NNV. Antiviral activity and specific antibody levels were determined in serum samples as well as the fish protection.

Conclusions

The vaccine elicited antiviral activity and antibody levels in sea bass specimens as well as increased the relative protection survival up to 57.9%. This is one of the few NNV vaccines tested in sea bass and the protection conferred was very similar to other NNV vaccine types and other fish species.

Acknowledgements. Financial support by grants AGL2013-43588-P and AGL2014-51839-C5-1-R (MINECO and FEDER) and 19883/GERM/15 (*Fundación Séneca de la Región de Murcia*, Spain) is gratefully acknowledged.

FEMS7-1680
Virology

HUMAN SAPOVIRUS IN STOOL SAMPLES OF SPAIN. A ONE YEAR STUDY

M. F. Varela[1], E. Rivadulla[1], J. L. Romalde[1]
[1]Universidade de Santiago de Compostela, Department of Microbiology and Parasitology, Santiago de Compostela, Spain

Backgrounds

Human Sapovirus is an emerging pathogen causing gastroenteritis in people of all ages worldwide. It has nonenveloped icosahedral morphology, small size (30-38 nm) and cup-shaped depressions on its surface. Sapovirus genome is a single-stranded positive-sense RNA molecule of 7.3-7.5 kb, with two or three open reading frames, depending on the genogroup. Its transmission occurs via the faecal-oral route. Symptoms are similar to those caused by others enteric viruses such as Norovirus, therefore laboratory diagnosis is needed to study its emergence.

Objectives

The main objective of the study was to determine the prevalence of human Sapovirus among the population of the Galician region (NW of Spain) through the analysis of samples from symptomatic outpatients with different ages and during different seasons of the year.

Methods

A total of 2,372 stool samples were collected weekly over one year, starting July 2010, from the Clinical University Hospital of the city of A Coruña, Galicia. Detection and quantification was carried out by RT-qPCR using specific primers and probes for human genogroups I, II and IV.

Conclusions

Overall detection rate was 14.3%, being slightly higher in ages 0-2 and 3-5 years old (15.6 and 15.2%, respectively). Positive samples were located along all months of the year, with peaks of detection on September, October and February. Mean value for quantified samples was 8.9×10^5 RNAc/g of feces, with the age group between 0-2 years old presenting the highest quantification and elders than 60 years old the lowest (5.6×10^6 and 1.3×10^5 RNAc/g of feces, respectively).

FEMS7-1997
Virology

THE SPREAD OF PICI-LIKE ELEMENTS IN GRAM-NEGATIVE BACTERIA

A. Fillol-Salom[1], R. Martínez-Rubio[2], R. Abdulrahman[1], R. P Novick[3], R. Davies[1], J. R Penades[1]
[1]Infection- Immunity and Inflammation, Bacteriology, Glasgow, United Kingdom
[2]Universidad CEU Cardenal Herrera, Departamento de Ciencias Biomédicas, Moncada, Spain
[3]Skirball Institute, Departments of Microbiology and Medicine, New York University Medical Center, USA

Backgrounds

Staphylococcus aureus pathogenicity islands (SaPIs) are mobile genetic elements (MGEs) with extreme importance in virulence intimately related to certain temperate (helper) bacteriophages, whose life cycles they parasitise. We have recently discovered and extensively characterized a family of pathogenicity islands in Gram-positive cocci, the phage-inducible chromosomal islands (PICIs), which contribute substantively to horizontal gene transfer, host adaptation and virulence.

Objectives

We now report that similar elements also occur widely in Gram-negative bacteria. Their uniqueness is defined by a constellation of features: unique and specific attachment sites, exclusive PICI genes, a phage-dependent mechanism of induction, unique replication origin organisation, convergent mechanisms of phage interference and specific packaging of PICI DNA into phage-like infectious particles, resulting in very high transfer frequencies.

Methods

By an *in silico* analysis we have identified a considerable collection of putative PICI-like elements in different Gram-negative bacteria.
Afterwards, we have performed *in vitro* and *in vivo* characterisation of the different modules. And finally to characterise the regulatory system we have used an enzymatic assays.

Conclusions

Overall, based on these findings we propose that all these elements along with the elements found in Gram-positive bacteria represent the discovery of a new and unique class of mobile genetic elements, the phage-inducible chromosomal islands, which have had a broad impact on lateral gene transfer in the bacterial world. Also, we suggest that the PICIs represent two or more distinct lineages, have spread widely throughout the bacterial world, and have diverged much more slowly than their host organisms or their prophage cousins.

FEMS7-0083
Virology

NATURAL VARIATION IN THE STABILITY OF SULFOLOBUS ISLANDICUS ROD-SHAPED VIRUSES ISOLATED FROM YELLOWSTONE NATIONAL PARK

K. Gazi[1], *D. Grogan*[1]
[1]*University of Cincinnati, Department of Biological Sciences, Cincinnati, USA*

Backgrounds

Among the three domains of cellular life, archaea are the least understood, and functional information about archaeal viruses is very limited. For example, it is not known whether many of the viruses that infect hyperthermophilic archaea retain infectivity for long periods of time under the extreme conditions of geothermal environments.

Objectives

To investigate these viruses' capability of Infecting under the extreme conditions of geothermal environments.

Methods

A number of plaque-forming viruses related to SIRVs, isolated from Yellowstone National Park in a previous study, were evaluated for stability under different stress conditions, including high temperature, drying, and extremes of pH.

Conclusions

Screening of 34 isolates revealed a 95-fold range of survival with respect to boiling for two hours and 94-fold range with respect to drying for 24 hours. Comparison of 10 viral strains chosen to represent the extremes of this range showed little correlation of stability with respect to different stresses. For example, three viral strains survived boiling but not drying. On the other hand, five strains that survived the drying stress did not survive the boiling temperature, whereas one strain survived both treatments and the last strain showed low survival of both. The basis for these differences has not been identified, but the extent of the variation suggests that multiple properties of each viral isolate combine to determine the biological stability of the virions.

FEMS7-1930
Virology

EXPRESSION ANALYSIS OF MIRNA IN PLASMA DURING AN INFECTIOUS SALMON ANAEMIA VIRUS INFECTION

C. Salazar[1,2], S.H. Marshall[1,2], F. Gómez[3]
[1]Pontificia Universidad Católica de Valparaíso, Biology Department, Valparaiso, Chile
[2]Fraunhofer Chile Research, Valparaíso, Chile
[3]Laboratorio de Genética e Inmunología molecular, Instituto de Biología,
Pontificia Universidad Católica de Valparaíso

Backgrounds

Infectious salmon anemia virus (ISAV) belongs to the *Orthomyxovirus* family and possesses a single stranded RNA genome of negative polarity divided into 8 segments. ISAV is the causative agent of the infectious salmon anemia disease which affects *Salmo salar,* causing a big outbreak in 2007 in Chile; since then the production recovered but the presence of the virus remains a problem.

miRNAs are small non-coding RNA which regulate mRNA expression at post-transcriptional level. They have been found in different body fluids, like plasma and serum, and their expression pattern can be altered in a variety of pathophysiological conditions.

Objectives

Given the importance of the salmon industry in Chile, a deep understanding of the interaction between ISAV and the target cell is a must. In the present work, we studied the expression pattern of miRNA on plasma samples during an ISAV infection on *S. salar* fishes.

Methods

Briefly, fishes were intraperitoneally infected with ISAV, and at 5 and 10 days post infection plasma samples were obtained from infected fishes and controls and small-RNA Seq was performed. The obtained data was analyzed using bioinformatic tools aiming to identify different expression of miRNA in plasma between control versus infected samples.

Conclusions

An *in silico* analysis of expression was performed and showed that three miRNA: miR-122, miR-29c and miR-145 are differentially expressed on infected plasma samples. As conclusion *S. salar* present miRNA on plasma and their pattern of expression changes during ISAV infection.

FEMS7-2097
Virology

COMPARATIVE ANALYSIS OF VARIATION AND SELECTION IN THE HCV GENOME

J.Á. Patiño-Galindo[1], F. González-Candelas[1]
[1]FISABIO-CSISP / University of Valencia, Joint Research Unit Infection and Public Health, Valencia, Spain

Backgrounds

Genotype 1 of the hepatitis C virus (HCV) is the most prevalent of the variants of this virus. Its two main subtypes, HCV-1a and HCV-1b, are associated to differences in epidemic features and risk groups, despite sharing similar features in most biological properties. Analyzing the selective pressures acting on these subtypes is interesting for antiviral research.

Objectives

To analyze the impact of positive selection and structural constraints on the evolution of HCV-1a and HCV-1b genomes and to compare the selective pressures in the different proteins of these two subtypes.

Methods

393 HCV-1a and 179 HCV-1b sequences were subjected to diversifying positive selection analyses with FEL and MEME. By means of logistic regression analyses, the distributions of positively selected and conserved sites were compared, in each subtype, considering different factors such as RNA secondary structure, the presence of different epitopes (antibody, CD4 and CD8), and secondary protein structure.

Conclusions

Less than 10% of the genome was found to be under positive selection, and purifying selection was the main evolutionary process acting in both subtypes. Logistic regression analyses revealed that similar selective forces act at the genome level in both subtypes: RNA secondary structure and CD4 T-cell epitopes are associated with conserved sites, while CD8 T-cell epitopes are associated with positive selection in both subtypes.
Similar selective constraints are acting along HCV-1a and HCV-1 b genomes. The results obtained give information about the effect of some interactions between HCV and its host on HCV variability, which may be useful for antiviral research against this virus.

FEMS7-2367
Virology

CHARACTERISATION OF NOVEL STAPHYLOCOCCUS AUREUS-INFECTING PHAGE

B. Grygorcewicz[1], A. Augustyniak[1], A. Wasak[1], X. Stachurska[1], J. Laskowska[1], P. Nawrotek[1]
[1]West Pomeranian University of Technology-
Szczecin- Faculty of Biotechnology and Animal Husbandry,
Department of Immunology- Microbiology and Physiological Chemistry, Szczecin, Poland

Backgrounds

Staphylococcus aureus is a hazardous etiological agent of skin and wound infections. Additionally, health problems caused by methicillin-resistant *Staphylococcus aureus* (MRSA) became a great challenge in terms of eradication of the pathogen. Bacteriophage-based therapy can be an effective method for treatment of the MRSA infections. Additionally, phage therapy can significantly reduce the cost of standard treatment based on antibiotics.

Objectives

The aim of this study was to isolate a novel *Staphylococcus aureus*-infecting bacteriophage and to evaluate its lytic activity.

Methods

Bacteriophage was isolated from bovine milk with *S. aureus* ATCC 6538 used as a host. The morphology of the bacteriophage was observed using transmission electron microscopy. The host range together with the efficiency of plaquing, adsorption rate, multiplication parameters, pH and thermal stability were evaluated by plaque assay based on the double agar layer method. The lytic activity of bacteriophage as well as the degradation of biofilm formed by *S. aureus* were assessed by spectrophotometric methods.

Conclusions

The isolated bacteriophage can effectively lyse planktonic cells and the degrade biofilm biomass of numerous *S. aureus* strains (n=87). This suggests that isolated bacteriophage could be applied as a therapeutic/biocontrol agent against *S. aureus* strains of human and animal origin.

FEMS7-2637
Virology

ISOLATION OF VIRUSES INFECTING HALOARCHAEA FROM URMIA LAKE

H. Hashemi Motahar[1], *E. Arefian*[1], *M.A. Amoozegar*[1]
[1]*Faculty of Biology - College of Science - University of Tehran -, Microbiology, Tehran, Iran*

Backgrounds

Viruses play an important role as a controller of the prokaryotic population as they are the only known predators in hypersaline environments. Moreover, they provide applicable genes and proteins to industries. All known archaeoviruses which are increasingly studied in the last decades, infecting two phyla *Euryarchaeota* and *Crenarchaeota*.

Objectives

Urmia Lake (located in the northwest of Iran) is one of the largest hypersaline lakes in the world and has faced with drought problem in recent years. The goal of this study was to identify the viral resources and protect them against destruction danger.

Methods

hypersaline water samples were collected from Urmia Lake and centrifuged. The sedimented phase was inoculated to MGM 23% media for isolating the archaea. The Antibiotic test was performed to approve the archaeal identity. To finding the culture logarithmic phase, the growth curves of the isolates were drawn using spectrophotometric experiment. The supernatant was passed through 0.45 and 0.22 µm syringe filters and was mixed to archaeal hosts and MGM soft agar and then poured to solid media for plaque assay. The plaques were isolated and viruses re-cultured several times to obtain purified plaques. The morphological features of the viruses were studied by Trans Electron Microscope (TEM).

Conclusions

TEM analysis revealed spherical morphology with an inner membrane surrounded by an icosahedral capsid.

FEMS7-2378
Virology

TRANSCRIPTOMICS AND PROTEOMICS OF THREE PHAGE INFECTIONS WITH VARYING EFFICIENCY

C. Howard-Varona[1], K.R. Hargreaves[1], N.E. Solonenko[1], L.M. Markillie[2], R.A. White III[2], C. Ansong[2], G. Orr[2], J.A. Adkins[2], M.B. Sullivan[1]
[1]The Ohio State University, Microbiology, Columbus, USA
[2]Pacific Northwest National Laboratory PNNL, Environmental Molecular Sciences Laboratory EMSL, Richland, USA

Backgrounds

Bacterial viruses (phages) are central to applied and environmental microbiology given their benefits in phage therapy and their impacts to cell mortality, gene transfer and biogeochemical cycles, respectively. Driving these are the strategies and outcomes of phage-host interactions, including host-range (generalist/specialist) and infection efficiency (efficient/inefficient), which are especially poorly understood in nature.

Objectives

Here we seek to understand infection strategies of a generalist and a specialist phage infecting one environmental *Bacteroidetes* host with opposite infection efficiency.

Methods

Genome-wide transcriptomics and proteomics, coupled with phage-host physiology experiments and genomics

Conclusions

Overall, phages had similar transcriptional dynamics, but their proteomes and different host response to each phage revealed mechanisms driving infection efficiency. Namely, controlling host transcriptome and proteome enabled the specialist phage to induce early activation of host translation and have an efficient infection by: *i*) expressing anti-restriction genes early and overcoming host restriction/modification defenses; and *ii*) synchronizing transcription and translation. The generalist's inefficient infection lacked such cellular take-over and failed to *i*) overcome host restriction/modification and protease defenses, and *ii*) synchronize transcription and translation, which resulted in delayed protein production. However, when infecting its original host, a genetically-related strain, the generalist phage displayed transcriptional-translational synchronization and the infection was efficient. Together, these data provide insight into generalist/specialist and efficient/inefficient infection strategies, and a foundation for investigating any phage-host interaction in nature.

FEMS7-2468
Virology

MULTIEPITOPE PROTEINS BASED ON THE ENVELOPMENT PROTEIN E2 OF CHIKUNGUNYA VIRUS AND THE NON-STRUCTURAL PROTEIN NS1 OF DENGUE VIRUS AS ANTIGENS TO DETECT VIRUS INFECTIONS

L.M.S. López[1], A. Rojas[1], M.E. Acosta[1], L. Aria[1], Y. De Guillén[1], B. Infanzón[1]
[1]Instituto de Investigaciones en Ciencias de la Salud- Universidad Nacional de Asunción, Production Department, Asunción, Paraguay

Backgrounds

Chikingunya virus (CHIKV), an emergent alphavirus during the last years, and dengue virus (DENV) an endemic flavivirus, are cause of many cases of fever in Paraguay. For this reason, it becomes necessary to develop a fast and accurate serological diagnosis of these infections.

Objectives

We aimed to produce two multiepitope recombinant functional proteins as antigens for antibody detection.

Methods

A synthetic gen was designed to express the mE2 based on the synthetic protein reported before. The gene was composed of four epitopes of the envelopment E2 of CHIKV. A second synthetic gene coding for a protein mNS1, derived from specific epitopes of the non-structural protein NS1 of the four DENV types, was designed after the study of isolated sequences reported in the country and the border countries. It is important to point that the DENV vaccine, commercialized since 2016, is based on the envelopment protein of the virus, therefore, the development of a test based on another antigenic protein, is crucial for the control of new cases after vaccination.

The two chimeric proteins, mE2 and mNS1, were expressed in *Escherichia coli* and were purified from crude extract by affinity chromatography. The antigenic role of the protein has been assayed by western blot with positives and negatives serum samples.

Conclusions

The production of the two proteins, mE2 and NS1, may allow the development of a local, low cost, and more sensitive test to improve the diagnosis during epidemic episodes

FEMS7-1070
Virology

GENETIC DIVERSITY OF HUMAN METAPNEUMOVIRUS IN HOSPITALIZED CHILDREN WITH ACUTE RESPIRATORY INFECTIONS IN CROATIA

M. Jagušić[1], A. Slovic[1], S. Ljubin Sternak[2], G. Milinarić-Galinović[3], D. Forčić[1]
[1]University of Zagreb, Centre for Research and Knowledge Transfer, Zagreb, Croatia
[2]University Medical School of Zagreb and Teaching Institute of Public Health "Dr. Andrija Štampar", Molecular Microbiology Department, Zagreb, Croatia
[3]Croatian National Institute of Public Health, Department of Virology, Zagreb, Croatia

Backgrounds

Human metapneumovirus (HMPV) is recognized as an important etiological agent of acute lower respiratory tract infections in infants.

Objectives

The objectives of this study were molecular epidemiology and evolutionary analysis of HMPV strains which produced moderate and severe acute respiratory tract infections in children in Croatia during four consecutive seasons (2011–2014).

Methods

A total of 117 HMPV samples collected from hospitalized paediatric patients presenting with acute respiratory tract infections and tested by direct immunofluorescence assay were analysed by amplifying a part of the F gene. Sixteen samples were further phylogenetically analysed based on sequences of all three virus transmembrane glycoproteins: F, G and SH gene.

Conclusions

HMPV genome was identified in 92 of 117 samples (78%); the circulation of multiple lineages of HMPV was confirmed. In 2011, 2012, and 2014, subgroups A2 and B2 co-circulated, while B1 gained prevalence in 2013 and 2014. Importantly, the presence of a unique subcluster A2c in Croatia was established. This study provides new insights into epidemiology and genetic diversity of HMPV in this part of Europe.

FEMS7-2801
Virology

TWO FATAL CRIMEAN-CONGO HEMORRHAGIC FEVER FOLLOWING INFECTION WITH AN AP92 LIKE STRAIN OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN IRAN

T. Jalali[1], M. Fazlalipour[1], V. Baniasadi[1], S. Azad-Manjiri[1], T. Mohammadi[1], Y. Jamshidi[1], S. Khakifirouz[1], M. Salehi-Vaziri[1]
[1]pasteur Institute of Iran, Arboviruses and Viral Hemorrhagic Fevers National Ref Lab, Tehran, Iran

Backgrounds

Crimean-Congo Hemorrhagic Fever (CCHF) is an important tick-borne viral zoonotic disease which can cause up to 50% mortality in infected patients. The main routes of transmission of CCHFV are by infected tick bites or through exposure to blood or tissues of patients or viremic animal. The disease is known to be endemic in Africa, South East Europe, Asia and the Middle East. Previously it has been proposed that the AP92 strain of CCHF virus is not virulent and could be used as a vaccine candidate; however, in recent years there have been reports of mild clinical diseases following infection with AP92 like strains.

Objectives

Here, we report two fatal cases of CCHF following AP92 like strains of CCHFV in north of Iran.

Methods

In summer of 2015, sera of two CCHF probable cases were sent to the Department of Arboviruses and Viral Hemorrhagic Fevers (National Ref Lab), Pasteur institute of Iran for CCHF laboratory diagnosis.

Molecular analysis of serum indicated CCHFV infection for both cases and serological analysis could not detect IgM or IgG in their samples. Sequence analysis revealed high similarity of these isolates to the AP92 strain. The isolates were designated as Iran-4465 and Iran-4675 with accession numbers KT588640 and KT899991 respectively.

Although both cases were subjected to ribavirin and supportive therapy, they died due to server hemorrhage.

Conclusions

Although AP92 strain of CCHF is not considered virulent in Greece and Turkey, interestingly we report fatal CCHF cases due to AP92 like strains. The AP92 like strains are genetically distinct from other strains reported so far in Iran.

FEMS7-2839
Virology

NOSOCOMIAL AND LOCAL OUTBREAK OF CRIMEAN CONGO HEMORRHAGIC FEVER IN IRAN

M. Salehi-Vaziri[1], V. Baniasadi[2], M. Fazlalipour[2], S. Khakifirouz[2], S. Azadmanjiri[2], T. Mohammadi[2], Y. Jamshidi[2], S. Azizizadeh-Haghighi[2], M.H. Pouriayevali[2], T. Jalali[3]
[1]Pasteur Institute of Iran, Arboviruses and Viral Hemorrhagic Fevers National Ref Lab, Tehran, Iran
[2]Pasteur Institute of Iran, Arboviruses and Viral Hemorrhagic Fevers National Reference Laboratory, Tehran, Iran
[3]Pasteur Institute of Iran, Arboviruses and Viral Hemorrhagic Fevers National Reference Laboratory, Tehrani, Iran

Backgrounds

Crimean-Congo hemorrhagic Fever (CCHF) is considered the main viral hemorrhagic fever (VHF) and arboviral disease in Iran. It is a vector-borne zoonotic disease transmited through infected tick-bites, nosocomially or contact with blood and tissues of viremic livestock.

Objectives

The objective of this work is to report nosocomial and local outbreaks of CCHF in Iran in recent years and to determine the risk factors.

Methods

According to guidline of National Expert Committee on VHFs in Iran (NEC), serum sample of CCHF probable cases are refered to Department of Arboviruses and VHFs (National Ref Lab), Pasteur Institute of Iran for molecular and serological analysis by RT-PCR and IgM ELISA.
From October 2000 to January 2017, 4586 probable cases had been analyzed in which 1179 were IgM and/or RT-PCR positive and among them 160 were fatal cases, during this period more than eight outbreaks of CCHF have occurred in Khorasan-e-Razavi, Khorasan-e-Shomali, Khuzestan, Fars, Sistan-va-Baloochestan and Yazd provinces with 30 confirmed cases. 21 cases (70%) were infected due to direct exposure to blood/tissues of viremic livestock and the remaining 9 cases (30%) became infected through direct contact with CCHF patients. In these outbreaks, 5 (17%) confirmed cases were fatal in which 2 were healthcare workers.

Conclusions

The analysis of CCHF outbreaks in Iran reviled that most important route of transmission is direct contact with infected blood/tissues of viremic livestock as well as nosocomial infections in Iran; Therefore, decrease in traditional slaughtering, development of standard abattoir and education of the healthcare workers for standard case management may play important role in prevention and control of the disease in Iran.

FEMS7-0815
Virology

NEXT GENERATION SEQUENCING (NGS) ANALYSIS REVEALS ATTENUATING MUTATIONS IN VARICELLA-ZOSTER VIRUS FOLLOWING EXTENSIVE IN VITRO PASSAGING

J.S. Jeon[1], Y.H. Won[1], S.C. Kim[1], J.H. Ahn[2], M.J. Song[3], O.S. Shin[4], C.H. Lee[1]
[1]Chungbuk National University, Microbiology, Cheongju, Republic of Korea
[2]Sungkyunkwan University School of Medicine, Molecular Cell Biology, Suwon, Republic of Korea
[3]Korea University, Biosystems and Biotechnology, Seoul, Republic of Korea
[4]Korea University, Biomedical Sciences, Seoul, Republic of Korea

Backgrounds

Varicella-zoster virus (VZV) is a causative agent for chickenpox in primary infection and shingles after reactivation from latency. Comparative genomic sequence analysis of clinical and vaccine strains suggested potential sites responsible for attenuation.

Objectives

In this study, we attempted to understand attenuating mutations in highly passaged VZV strains.

Methods

Three clinical VZV strains were passaged *in vitro* cell culture up to 110 times. Different passages of YC01 (p14, p61, p110), YC02 (p14, p61, p110) and YC03 (p6, p61, p110) were subjected to next-generation sequencing and their full genome sequences were compared with each other.

Conclusions

Mutations were detected at 300, 256, and 126 sites in the strains YC01, YC02 and YC03, respectively. Most of the mutations were A to G and T to C transitional mutations. Approximately 70~80% of mutations were found in open reading frames (ORF), and ORF 62 exhibited highest frequency of mutations. Codon adaptation index (CAI) values decreased while %GC values increased as VZV strains were passaged *in vitro* cell culture. Vaccine-type mutations were found at 7 positions, T to C substitution at 5 positions (560, 105705, 106262, 107252, 108111) and A to G substitution at 2 positions (105169, 111650). Direct PCR sequencing of these positions at various passages of VZV strains identified the passage numbers when the attenuating mutations occurred. Thus, our study provides new insight into the extent of the attenuating mutations associated with *in vitro* passaging of multiple VZV strains.

FEMS7-1313
Virology

IDENTIFICATION OF INTERLEUKIN-28B POLYMORPHISMS IN THE HIGH SUSTAINED VIROLOGICAL RESPONSE OF INDONESIAN CHRONIC HEPATITIS C TO PEGYLATED-INTERFERON/RIBAVIRIN TREATMENT

J. Juniastuti[1], B. Wibowo[2], I. Wibawa[3], T. Utsumi[4], S. Mustika[2], M. Amin[5], R. Wahyuni[5], H. Kurniawan[5], A. Hendrayana[6], L. Yamani[5], S. Soetjipto[7], Y. Yano[4], H. Hotta[4], Y. Hayashi[4], M. Lusida[8]
[1]Airlangga University, Department of Microbiology- School of Medicine, Surabaya, Indonesia
[2]Dr. Saiful Anwar General Hospital, Department of Internal Medicine, Malang, Indonesia
[3]Sanglah General Hospital, Department of Internal Medicine, Denpasar, Indonesia
[4]Kobe University Graduate School of Medicine, Center for Infectious Diseases, Kobe, Japan
[5]Airlangga University, Institute of Tropical Disease, Surabaya, Indonesia
[6]Udayana University, Department of Microbiology- School of Medicine, Denpasar, Indonesia
[7]Airlangga University, Department of Biochemistry- School of Medicine, Surabaya, Indonesia
[8]Airlangga University,
Department of Microbiology- School of Medicine and Insitute of Tropical Disease, Surabaya, Indonesia

Backgrounds

The used standard-of-care treatment for chronic hepatitis C is Pegylated-Interferon/Ribavirin (PEG-IFN/RBV), however, many patients do not achieve a sustained virological response (SVR). It has been known that the success of this treatment is affected by viral and host factors. Recent studies revealed a strong association between Interleukin-28B (IL-28B) single-nucleotide polymorphisms (SNPs) and the response, which varied among ethnic groups.

Objectives

This study aimed to investigate the IL-28B SNPs associated with the HCV treatment response among HCV genotypes in Indonesians.

Methods

IL-28B SNPs were analyzed by PCR-direct sequencing in PBMC of 34 treated patients in Malang and Denpasar, Indonesia. HCV genotype and viral load were also examined.

Conclusions

Most patients were ethnic Java people (82.4%) and the rest were Bali, Batak-Lampung, Java-Madura, Gorontalo, Japan-Toraja people. They were mostly infected with HCV genotype 1 (70.6%), followed by genotypes 2 and 3. However, they mostly achieved EVR (Early Virological Response)/SVR (75.8%). Analysis of IL-28B SNPs revealed that most patients (94%) carried major genotypes of rs12979860 (CC), rs11881222 (TT), rs8103142 (AA), rs8099917 (TT), except one patient with heterozygous genotypes of the four SNPs and another patient with the major genotypes of rs12979860, rs11881222, rs8099917, but heterozygous genotype of rs8103142. Most patients with the major genotypes (75.0%-75.8%) and one patient with the major genotypes of the three SNPs achieved EVR/SVR, whereas the other patient with heterozygous genotypes of the four SNPs had NVR (Non-Virological Response).
In conclusion, the IL28B SNPs in Indonesian patients (mostly Javanese) were mainly the major genotypes, which is considered to be advantageous for PEG-IFN/RBV.

FEMS7-0688
Virology

ISOLATION OF HALOPHILIC AND HALOTOLERANT BACTERIA INFECTING BACTERIOPHAGE IN SOILS AND SEDIMENTS OF URMIA SALT LAKE

R. Kamyab[1], E. Arefian[1], M.A. Amoozegar[1]
[1]Faculty of Biology - College of Science - University of Tehran -, Microbiology, Tehran, Iran

Backgrounds

soil microorganisms are essential for nutrient cycle and life of ecosystems. Many studies show they are playing an important role in evolution of their bacteria. The Urmia Lake is third largest saline lake in Iran. These years due to drought and loss of many biological species in this lake, identification and conservation of lake genetics resources is critical.

In this study soil and sediment from southern part of lake were collected and transferred to the laboratory and according to culture-dependent approaches, bacteria and phages were isolated. Bacterial hosts were identified R and plaque isolated bacteriophages were characterized by transmission electron microscopy.

Objectives

The aim of this study was isolation and characterization of phages from Urmia Lake halophilic and halotolerant bacteria for preservation of lake genetic resources.

Methods

serial dilutions of soil and sediments samples were cultured on SWN medium and incubated at 34° C for 24 hours. Bacteria colonies were isolated and purified. Molecular identification of bacterial microorganisms was performed by 16srRNA sequencing. Then fifty grams of soils and sediments inoculated in SWN broth medium and incubated for 24 h at 34 °C on a shaking platform. The supernatant was separated by centrifugation and passed through 0/45µm and 0/22µm filters respectively. To perform a plaque assay, the filtrated lysate was inoculated onto hosts. After plaques isolation, phages were purified with spot assay and morphological analysis was performed by negative staining and TEM.

Conclusions

TEM figures showed Purified bacteriophage**s** are belong to head-tail viruses. The morphological analysis propose that virus is related to *Myoviridae* family.

FEMS7-2817
Virology

IDENTIFICATION OF VACCINIA VIRUS REPLISOME PROTEINS BY IPOND COUPLED WITH MASS SPECTROMETRY

T. Senkevich[1], *B. Moss*[1]
[1]*National Institute of Allergy and Infectious Diseases, Laboratory of Viral Diseases, Bethesda, USA*

Backgrounds

Vaccinia virus (VACV) replicates in the cell cytoplasm and encodes most of the proteins needed for its autonomous life cycle.

Objectives

The major requirements for DNA replication are fulfilled by 9 virus proteins but uncertainty remains as to whether this set is complete and whether host proteins might supply some missing functions.

Methods

To address this question, we applied the recently developed iPOND (Isolation of Proteins on Nascent DNA) method that has been proven a valuable tool for the identification of protein composition of eukaryotic replisomes.

Conclusions

We adjusted iPOND for the analysis of the cytoplasmic VACV replisome and confirmed the association with pulse-labeled DNA of all 9 VACV proteins shown to be required for DNA replication. In addition, the telomere-binding protein I1 that was not previously implicated in DNA replication was identified as a replisome component. Among host proteins, both subunits of topoisomerase II were confidently identified in the replisome from wild-type infected cells but not from cells infected with a knockout in VACV DNA ligase gene, in agreement with the previously reported interaction of viral ligase with topoisomerase II. Other host proteins involved in DNA replication or repair were virtually absent from VACV replisomes, with a possible exception of PCNA. Additionally, 16 VACV proteins involved in intermediate and late but not early transcription were also detected in association with pulse-labeled DNA. These findings suggest coupling of DNA replication with intermediate and late transcription as was recently shown for herpes simplex virus.

FEMS7-2464
Virology

STRUCTURAL BIOLOGY OF THE GENETIC SWITCH FROM BACTERIOPHAGE TP901-1

K.K. Rasmussen[1], K.E.H. Frandsen[1], E.B. Erba[2], M. Pedersen[3], A.K. Varming[1], M. Kilstrup[4], K. Hammer[4], P.W. Thulstrup[1], M.R. Jensen[2], L. Lo Leggio[1]
[1]University of Copenhagen, Department of Chemistry, Copenhagen, Denmark
[2]University Grenoble Alpes, Institut de Biologie Structurale, Grenoble, France
[3]Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark
[4]Technical University of Denmark, Metabolic signalling and regulation- Department of Bioengineering, Lyngby, Denmark

Backgrounds

Virus particles infecting bacteria, bacteriophages, can be classified into two categories namely lytic or temperate bacteriophages. TP901-1 is a temperate bacteriophage, and infects *Lactococcus lactis*. TP901-1 either produce new progeny (lytic life cycle), or survive as prophage in the infected bacterium (lysogenic life cycle). A genetic switch consisting of two promoters, three operator sites (O_R, O_L and O_D), and two proteins CI and MOR, controls the lysis-lysogeny decision. The hexameric repressor CI, binds to O_R, O_L and O_D, resulting in the lysogenic life cycle, whereas MOR modulates the repression in a so far unknown way, leading to the lytic life cycle.

Objectives

We are investigating the structural biology of this genetic switch by complementary biochemical and biophysical techniques.

Methods

Includes X-ray crystallography and solution scattering, mass spectrometry, CD and NMR spectroscopy.

Conclusions

We have determined the crystal structure of the DNA binding domain (NTD) of CI in complex with the O_L half site and characterized a dimeric, truncated variant of CI (CIΔ58) showing cooperativity of DNA binding for the dimer and identifying a helical dimerization region linked to the NTD by a flexible linker. Recently we have modelled the CIΔ58:O_L complex based on SAXS data. Our current focus now are determination of the structure of the C-terminal multimerization region of CI by crystallography, and the interaction of CIΔ58:MOR by native mass spectrometry, NMR spectroscopy and SAXS. Initial results suggest that CIΔ58:MOR and CIΔ58:O_L complexes are mutually exclusive supporting the hypothesis of a competition between CI and MOR as determinant of the cell fate.

FEMS7-0155
Virology

SEVEN AMINO ACID DIFFERENCES IN 126 KDA AND MOVEMENT PROTEIN (MP) BETWEEN TWO PMMOV ISOLATES AFFECTS QUALITY OF SEEDS AND SYMPTOM SEVERITY IN CAPSICUM ANNUUM

H. Lim[1], J.K. Kim[1], E.Y. Seo[1], S.H. Yu[1], J. Hammond[2]
[1]Chungnam National University, Applied Biology, Daejeon, Republic of Korea
[2]United States Department of Agriculture – Agricultural Research Service US National Arboretum, FNPRU, Beltsville, USA

Backgrounds

Two isolates of *Pepper mild mottle virus* (PMMoV) were selected from a nationwide survey of pepper fields in Korea from 2014 to 2015, in which *Cucumber mosaic virus* was also detected; the two PMMoV isolates, S-47 (KX399390) and J-76 (KX399389), share ~99% amino acid identity and are closely related to PMMoV-CN (AY859497), PMMoV-BJ (HQ699080) and PMMoV-J (HQ699080). Amino acid sequence comparisons revealed 99.75%, 99.82%, 98.51%, and 100% identity in the ORF1 (126 kDa), ORF22 (180 kDa), MP, and CP respectively in two isolates; there are three amino acid differences (R(142)K, D(583)K, V(931)I) in the 126 kDa protein and four (K(134)R, V(192)A, N(226)D, L(250)S) in MP.

Objectives

In order to compare seed quality and symptom severity of two isolates we generated infectious clones of S-47 and J-76, and T7 promoter driven transcripts.

Methods

Nicotiana benthamiana infected with the two isolates produced very severe symptoms, whereas only mild symptoms developed in *Capsicum annuum*. Differences in seed production and seed transmission between the two isolates were detected in *Capsicum annuum*, which might be the result of about thirteen times higher viral replication of J-76 than S-47. Gene silencing suppressor function of 126 kDa and cytoskeleton-connected plasmodesmata localization of MP of S-47 and J-76 showed no difference between isolates, whereas 126 kDa of S-47 clearly formed intranuclear aggregates not observed with J-76 126 kDa.

Conclusions

Differences between these isolates in RdRp-related functions such as replication and subcellular localization suggest that variation in replication efficiency or differential interactions with host proteins may affect seed production and seed transmission in *C. annuum*.

FEMS7-1492
Virology

SSRA-ASSOCIATED PROPHAGES IN SHEWANELLA

X. Liu[1]
[1]*South China Sea Institute of Oceanology, Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangzhou, China*

Backgrounds

Gene *ssrA* (tmRNA) is a hot spot for the integration of horizontally transferred genetic elements in bacteria.

Objectives

Through comparative genomic analysis, we found that 17 out of 54 *Shewanella* strains harbor prophages at *ssrA*.

Methods

Further analysis showed that these *ssrA*-associated prophages in *Shewanella* can be divided into two major groups, the P4-family group and the P2-family group. All of the P4-family prophages contain at least one copy of the *alpA* gene which is known to cause the P4-like prophage excision in *S. oneidensis* and in *E. coli*. To study whether P2-family prophages in *Shewanella* excite proficiently like P4-family group prophage, the gene contents of P2-family prophages were analyzed. Result showed that gene contents of the P2-family prophages are conserved in structural region and regulatory region. The regulatory region contains three adjacent regulatory proteins (CI, CII, and Cox). Though Cox protein in P2-like prophages in *Shewanella* (66 amino acids) lacks the C-terminus compared to the Cox of phage P2 (91 amino acids), expression of Cox protein in *Shewanella putrefaciens* W3-18-1 caused the excision frequency of *P2-like ssrA*-associated prophage increased 10^5-fold. Excision of *ssrA*-associated prophage in W3-18-1 abolish the SsrA function by destroy the G•U wobble base pair of *ssrA*, similar as the excision of P4-like prophage inserted in SsrA.

Conclusions

We found two major groups of prophage integrate at *ssrA* in *Shewanella*, P4-family group and P2-family group. Like AlpA induces P4-family prophage excision, Cox promotes excision of the P2-family prophage. Excision of P2-family prophage in W3-18-1 also abolish the *ssrA* gene function.

FEMS7-1715
Virology

NEW INSIGHTS INTO SPATIO-TEMPORAL DYNAMICS OF EUROPEAN BAT LYSSAVIRUS TYPE-1 IN EPTESICUS SP BATS

P. Mingo-Casas[1], V. Sandonís[1], E. Obón[2], J.M. Berciano[1,3], S. Vázquez-Morón[1], J. Juste[4], J.E. Echevarría[1,3]
[1]Centro Nacional de Microbiología - Instituto de Salud Carlos, Virology, Majadahonda, Spain
[2]Centro de Fauna Salvaje de Torreferrussa, Generalitat de Catalunya, Torreferrussa- Santa Perpètua de Mogoda, Spain
[3]CIBER, Epidemiology and Public Health CIBERESP, Madrid, Spain
[4]Doñana Biological Station CSIC, Evolutionary Ecology, Seville, Spain

Backgrounds

Rabies is an ancient zoonosis caused by several lyssaviruses throughout the world. One of them is the European Bat Lyssavirus type-1 (EBLV-1), which is endemic in great part of Europe and comprises two different subtypes (EBLV-1a and EBLV-1b). This virus is mainly associated to *Eptesicus serotinus*, which accounts for more than the 95% of the infected bats detected in Europe, including the northern half of the Iberian Peninsula (recently discovered). However, other sibling species, *Eptesicus isabellinus* accounts for all the cases detected in the south.

Objectives

Here, the spatio-temporal dynamics of EBLV-1 on this two species is studied in the general European context with the latest discovered strains.

Methods

A total of 185 EBLV-1 400 bp nucleoprotein sequences from all across Europe were analysed by Bayesian phylogenetic inference using the BEAST software package. Temporal dynamics were reconstructed by using a relaxed uncorrelated molecular clock, either with constant and exponential growth priors on population size to generate maximum clade credibility (MCC) trees.

Conclusions

Most recent common ancestor (tMRCA) estimation for EBLV-1 is considerably recent (175 years, 95% HPD: 47-260 years), quite higher to the tMRCA previously shown by Hughes, G.J 2008, but with lower 95% HPD interval. tMRCA estimates for viral lineages EBLV1a and EBLV1b were 70 and 55 years respectively, showing 15 years of difference between their ancient slice point in Europe. Thereby, we suggest a bottleneck pressure during XIX century to have extinct ancient EBLV-1 strains and influenced their *E. serotinus* hosts.

FEMS7-1957
Virology

CHARACTERIZATION OF PORCINE PICORNAVIRUSES (TESCHOVIRUS, SAPELOVIRUS, ENTEROVIRUS G) IN THE CZECH REPUBLIC

R. Moutelikova[1], *J. Prodelalova*[1], *M. Toman*[2]
[1]*Veterinary Research Institute, Virology, Brno, Czech Republic*
[2]*Veterinary Research Institute, Immunology, Brno, Czech Republic*

Backgrounds

Members of the family *Picornaviridae* are important viruses responsible for a variety of human and animal diseases. Porcine picornaviruses (namely Teschovirus A - PTV, Sapelovirus A - PSV and Enterovirus G – EV-G) are often found in apparently healthy pigs but they are capable of causing variety of disorders from mild diarrhoea to fatal encephalomyelitis (strain PTV-1). However, the pathogenicity and the ensuing importance of porcine picornaviruses in pig farming was not yet clearly defined.

Objectives

In our project an epizootiological study was carried out during which both healthy and diseased pigs of all age categories were sampled. First, the prevalence of monitored picornaviruses was determined. Second, the suitable tools for genotyping of PTV-, PSV-, and EV-G-positive samples were tested.

Methods

During the first year a collection of 122 faecal samples and 141 oral and nasal swabs were screened for the presence of picornaviruses with the reverse transcription-nested-PCR (RT-nPCR) with the use of primers detecting the conserved 5‘-NTR region of genome. For the purpose of genotyping of positive samples, partial nucleotide sequence of VP1 gene coding capsid protein was sequenced and compared to data in GenBank.

Conclusions

So far, in the collection of faecal samples there were 18 samples (14.8%) positive for PTV, 10 samples (8.2%) positive for PSV and 52 samples (42.6%) positive for EV-G. In 19.7% of samples more than one pathogen was found. In the set of oral and nasal swabs 16 samples (11.3%) were positive for PTV and 1 sample (0.7%) was positive for PSV.

FEMS7-0787
Virology

TARGETED DELIVERY OF PHAGES INDUCED ANTI-CANCER EFFECT IN MICE MODELS

H. Myung[1], M. Kim[1], H. YoonJung[1]
[1]Hankuk University of Foreign Studies, Bioscience and Biotechnology, Yongin, Republic of Korea

Backgrounds

Oncolytic animal viruses are well documented and proven to be effective against cancer. Their anticancer effect was generally based on two ways; one is their ability to infect and lyse host cells while the other is enhancement of host immune responses.

Objectives

Phages are viruses and can be engineered to dsiplay peptides targeting specific cancer cells. We wanted to see whether phages can act as oncolytic virues.

Methods

In this experiment, bacteriophage T7 was engineered to display target specific peptides against mouse tumor cell lines. Two different cell lines used were CT-26 (colorectal cancer) and B16-F10 (melanoma). Two peptides targeting each cell lines were TCP1 and pep42, respectively. Mice were grafted intraperitoneally with each cell line, and tumor mass was allowed to grow for 6 days. Then phage T7 displaying targeting peptides were injected into tumor mass and mice were further observed until day 17.

Conclusions

Mice grafted with CT-26 showed 85% reduction in tumor mass when treated with phages. Mice grafted with B16-F10 showed 76% reduction in tumor mass when treated with phages. Cytokines IL1-a and TNF-a increased significantly in mice treated with phages. Macrophage infiltration into tumor mass was observed from immunohistochemistry. Thus phage treatment could be another option as oncolytic viruses for cancer.

FEMS7-0816
Virology

INHIBITION OF HEPATITIS C VIRUS RNA BY SMALL RNAS DERIVED FROM TRNAS

H. Cho[1], W. Lee[1], G.W. Kim[1], S.H. Lee[1], J.S. Moon[1], M. Kim[1], J.W. Oh[1]
[1]Yonsei University, Biotechnology, Seoul, Republic of Korea

Backgrounds

Hepatitis C virus (HCV) propagation is dynamically regulated during the course of chronic infection that leads to liver diseases such as hepatocellular carcinoma (HCC) because cellular transcriptomes, including mRNAs and non-protein coding RNAs, which modulate proviral or antiviral host factors are subjected to reprogramming. Recent deep sequencing of small RNA libraries from various human cancer tissues and cell lines has identified diverse types of tRNA-derived RNA fragments (tRFs). Like microRNAs (miRNAs), tRFs may have diverse physiological roles in gene expression regulation, oncogenesis, and development.

Objectives

Despite accumulating evidence strengthening the notion of the presence of tRFs in cells, potential roles of tRNA-derived smRNA in host cells and during the course of pathogen infection remain elusive. Recently, tRFs, which were previously regarded as merely degradation intermediates, have emerged as potential effectors participating in gene expression regulation.

Methods

We analyzed small RNA profile in the human HCC cell line Huh7, which supports the propagation of HCV, and identified a 19-nt long structured, single-stranded RNA, with a 5′-monophosphate, as the most abundant tRF among single gene-mapped tRFs.

Conclusions

We provide evidence for a negative regulatory role of tRF_U3_1 on HCV, establishing a new paradigm for understanding pathophysiological roles of tRFs during the course of HCV infection.

FEMS7-3171
Virology

DESIGN AND DEVELOPMENT OF CHIKUNGUNYA VIRUS E2 RECOMBINANT ANTIGEN FOR CHIKV DIAGNOSTICS BASED ON GENETIC DIVERSITY ANALYSIS

C. Park[1], S.Y. Cho[2], S.H. Kim[2], S.H. Park[2], S.M. Choi[2], J.H. Choi[2], J.H. Yoo[2], D.G. Lee[2]
[1]The Catholic University of Korea - College of Medicine, Vaccine Bio Research Institute, Seoul, Republic of Korea
[2]The Catholic University of Korea - College of Medicine, Internal Medicine, Seoul, Republic of Korea

Backgrounds

The spread of mosquito-borne viral disease is increasing public concerns due to climate change and viremic travelers, even if not epidemic country. Chikungunya virus (CHIKV) is an alphavirus causing mosquito-borne viral disease characterized by high fever and sudden onset of polyarthralgia. Although there have been few report excepts for overseas travelers infected by CHIKV in South Korea, the development of diagnostics is an urgent need for the surveillance of these infectious disease.

Objectives

Based on the genetic analysis, we tried to design and develop E2 recombinant proteins which cover antigen variation for CHIKV diagnostics by using global gene database.

Methods

Two hundred fifty eight of E2 were analyzed by position-specific iterated BLAST. Ranged from 100 to 93.6%, ninety three of genetic variant were extracted, and their antigenic epitopes were analyzed by Kolaskar & Tongaonkar methods and Bepipred epitope prediction at IEDB. Among twenty epitopes in E2, 13 of variable region were found. Their codons were optimized for expression of *E. coli* Lemo (DE3) strains.

Conclusions

Phylogenetic analysis exhibited 4 major groups representative for geographic clonal lineage in E2 genes. Especially three of genetic variant were found in STKDNF (E2EP3 epitope) relevant to early neutralizing response to CHIKV. These candidates for diagnostics were purified in *E. coli* system by SDS denaturing method (1.887mg/L, 1.296mg/L, and 1.531mg/L respectively), and their antigenicity was confirmed by Western-blot. This study showed a trial of genetic design based on global database and an approach for coping with variations in viral diagnostics.

FEMS7-1597
Virology

THE NS3/NS4B REGION OF DENGUE VIRUS IS A DETERMINANT FOR EFFICIENT REPLICATION

S. Phanthanawiboon[1]
[1]*Faculty of Medicine/Khon Kaen University, Microbiology, Khon Kaen, Thailand*

Backgrounds

Varieties of dengue replication have been occurred from high to low due to genetic variation of RNA viruses. Replication capacity determines viral characters which contribute to viral virulence, host susceptibility and virus evolution. Both structural and non-structural proteins of dengue have been found to involve in replication process but theirs role in replication efficiency is not clear.

Objectives

This study aims to identify the protein of dengue serotype 4 which is essential for viral replication efficacy by using chimeric viruses.

Methods

Total 5 chimeric viruses (C-NS1, NS3-NS5, NS3-NS4B, NS4B-NS5, NS5) were constructed based on the restriction enzyme sites from high replicative (Dengue 4, H241) and low replicative (Dengue 4, R05-167) infectious plasmids. Replication ability of each chimeric viruses was determined in Vero and C6/36 mosquito cell line using focus and plaque forming assay. Essential amino acid of the high replicative NS3-NS4B chimeric was determined by single and double amino acid reverse-substitution.

Conclusions

We found the important role of NS3, NS4A and NS4B proteins of DENV4 in replication efficiency. Substitution of these proteins ameliorated replication ability and the size of virus. Only double amino acid reverse substitution affected viral replication of NS3-NS4B chimeric viruses revealed the important of amino acid combination in viral replication efficacy. These data provides useful information for further understanding of viral fitness and viral protection.

FEMS7-1996
Virology

PHAGE-INDUCIBLE CHROMOSOMAL ISLANDS IN THE GRAM-POSITIVE COCCI

N. Quiles-Puchalt[1], R. Martínez-Rubio[2], M. Martí[3], S. Humphrey[1], G. Ram[4], D. Smyth[4], J. Chen[4], R. Novick[4], J.R. Penadés[1]
[1]Infection- Immunity and Inflammation. University of Glasgow, Bacteriology, Glasgow, United Kingdom
[2]Universidad Cardenal Herrera, Departamento de Ciencias Biomédicas, Valencia, Spain
[3]Instituto valenciano de Investigaciones Agrarias CITA-IVIA, Centro de Investigación y Tecnología Animal, Castellón, Spain
[4]Skirball Institute. New York University Medical Center, Departments of Microbiology and Medicine, New York, USA

Backgrounds

The *Staphylococcus aureus* pathogenicity islands (SaPIs) are a cohesive subfamily of extremely common phage-inducible chromosomal islands (PICIs) that generally encode for genes involved in virulence and factors involved in the host adaptation process. They reside quiescently at specific attachment sites in the staphylococcal chromosome and are induced by helper phages to excise and replicate. They are usually packaged in small capsids composed of phage virion proteins, giving rise to very high transfer frequencies, which they enhance by interfering with helper phage reproduction.

Objectives

As the SaPIs represent a highly successful biological strategy, being widely distributed with many natural *Staphylococcus aureus* strains containing two or more, we search for similar elements in the Gram-positive cocci.

Methods

To identify and characterize similar elements in other Gram-positive cocci we have made use of BLAST searches and ortholog analysis along with different molecular techniques such as enzymatic assays, Southern blotting, and allelic mutant replacement.

Conclusions

Based on resemblance to the paradigmatic SaPI genome, we have identified large cohesive families of similar elements in the lactococci and pneumococci/streptococci plus a few such elements in *Enterococcus faecalis*. We have characterized in depth the enterococcal element, EfCIV583, and have shown that it very closely resembles the SaPIs in functionality as well as in genome organization. Furthermore, based on extensive ortholog analyses, we found that the PICI elements represent distinct but parallel lineages, suggesting that they represent convergent evolution towards a highly successful lifestyle. In summary, our findings greatly broaden the PICI family to include elements from three genera of cocci.

FEMS7-0629
Virology

ROLE OF AICHI VIRUS IN ACUTE GASTROENTERITIS IN GALICIA (NW SPAIN)

E. Rivadulla Veira[1], *M. Fernández Varela*[1], *J. López Romalde*[1]
[1]*University of Santiago de Compostela, Microbiology and Parasitology, SANTIAGO DE COMPOSTELA, Spain*

Backgrounds

Enteric viruses are the cause of the majority cases of acute gastroenteritis worldwide. They multiply in the intestines, are excreted in faeces and be dispersed through fecal-oral route. In recent years Aichi virus (AiV) has emerged as responsible for food-borne outbreaks associated with diarrheal disease.

Objectives

The aim of this study was the detection and quantification of AiV in clinical samples collected during a 1-year period (July 2010–June 2011) from Complejo Hospitalario Universitario de A Coruña (CHUAC).

Methods

A total of 1751 from outpatient specimens of all ages affected with gastroenteritis were examined using a reverse transcription-quantitative PCR (RT-qPCR) procedure.

Conclusions

AiV was detected in 47 samples (.3%). Positives percentage of the six different age-groups, that have been established for subsequent data analysis were: 0.87% from 0–2 years , 0.17% from 3–5 years, 0.29% from 6–12 years, 0.06% from 13– 18 years, 0.69% from 19–59 years , 0.58% from >60 years and 0.06% from seven samples of unknown age that were also included. These samples were negative for the principal agents of acute gastroenteritis worldwide as Rotavirus (RV), Norovirus (NoV) and Sapovirus (SaV), except one sample in which coinfection with NoV was found.

Evidences obtained indicate that the prevalence of this enteric virus is considerably lower than those of RV, NoV and SaV, but is still present at some extent in the populations. Further studies are needed in order to determine the actual role of AiV as a causative agent of acute gastroenteritis in Galicia.

FEMS7-2239
Virology

PHYLOGENETIC ANALYSIS OF BOVINE VIRAL DIARRHEA VIRUSES ISOLATED FROM CLINICAL INFECTED DAIRY COWS

N. Rudan[1], E. Marković[2], L. Barbić[1], Z. Hajek[3]
[1]Faculty of Veterinary Medicine University of Zagreb,
Department of Microbiology and Infections Diseases with Clinic, Zagreb, Croatia
[2]Ministry of Agriculture, Board of Veterinary Managers, Zagreb, Croatia
[3]Nexe group, Farm of dairy cows, Feričanci, Croatia

Backgrounds

In this research a BVDV infection was studied in the herd of dairy cattle, on the farm with 1500 Holstein-Friesian cattle, on which clinical symptoms characteristic for BVD were previously recorded. Animals in the herd were not vaccinated against BVDV, but were vaccinated against coronavirus, adenovirus and all bovine common respiratory infections.

Objectives

The aim of research was to detected the presence of BVD specific antibodies and also BVD viral antigens, with reference to viral nucleic acids in searched sera samples, because of evidence the persistent infections in the herd.

Methods

Prevalence of specific antibodies was proven using virus-neutralisation test (VNT) and Ab-ELISA test. Presence of antigens were tested by Ag-ELISA test, and viral nucleic acids with qRT-PCR method.

Conclusions

Presence of BVD antibodies was found in 194 out of 233 tested sera (83.26%) by VNT. With Ab-ELISA presence of BVD antibodies was found in 203 sera of tested cows (87.12%). Presence of antigens were proven in 2 of 233 sera samples (0.86%), and also confirmed by quantatitive PCR test. Phylogenetic analysis of BVD virus isolates, based on a comparative analysis of nucleotide sequences of 5'NTR segment, refer their affiliation to *genotype 1*, *subtype 1d* in phylogenetic tree.

FEMS7-0129
Virology

NEW HIGHLY IMMUNOGENIC VARIANTS OF ATTENUATED VACCINIA VIRUS

S. Shchelkunov[1], S. Yakubitskiy[1], I. Kolosova[1], R. Maksyutov[1]
[1]State Research Center of Virology and Biotechnology VECTOR, Genomic research, Koltsovo, Russia

Backgrounds

Since 1980, in the post-smallpox vaccination era, the human population has become increasingly susceptible to not only variola (smallpox) virus, but also other zoonotic orthopoxviruses. The need for new safe vaccines against orthopoxviruses is great now.

Objectives

The aim of this work was to introduce targeted deletions into the genome of vaccinia virus (VACV) as well as to study the reproductive properties of the resulting VACV strains in cell cultures and the level of induced virus neutralizing antibodies in vaccinated mice and the effectiveness of their protection against ectromelia virus (ECTV) infection at a lethal doses.

Methods

The Lister vaccine strain LIVP of VACV was used as a parental virus for generating a recombinant LIVPdelta5 clone defective in five virulence genes *A56R*, *B8R*, *J2R*, *C3L*, and *N1L* and LIVPdelta6 clone with an additional deletion in the *A35R* gene. Targeted disruption of these loci did not affect virus replication in mammalian cell cultures. Recombinant VACVs exhibited a reduced inflammatory response and attenuated neurovirulence relative to LIVP. In a subcutaneous mouse model, the level of virus neutralizing antibodies in the case of immunization with LIVP or LIVPdelta5 strains reached approximately the same level, which provided similar values of protection against ECTV. Strain LIVPdelta6 induced a significantly higher level of virus neutralizing antibodies and protection against ECTV infection compared to other VACV strains studied.

Conclusions

The LIVPdelta5 and LIVPdelta6 recombinant strains hold promise as a safe live vaccine strains for preventing smallpox and other orthopoxvirus infections.

FEMS7-0523
Virology

EFFICIENT INDUCTION OF IMMUNE RESPONSES BY DNA VACINE AGAINST ZIKA VIRUS

S. Shin[1], N.Y. Kim[2], H.B. Ahn[3], Y.K. Shin[3]
[1]Seoul National University, Bio-MAX/N-Bio, Seoul, Republic of Korea
[2]ABION Inc., Drug Development, Seoul, Republic of Korea
[3]Seoul National University, College of Pharmacy, Seoul, Republic of Korea

Backgrounds

Zika virus is an arbovirus of the Flavivirus genus within the Flaviviridae family. Recently, Zika virus infection has become public health concern following a large outbreak in South America in 2015. Due to the potential burden of illness from microcephaly and Guillian-Barre syndrome, WHO declares a global health emergency over Zika virus on February 1, 2016. Currently, no vaccine or antiviral therapy is available for Zika virus infection.

Objectives

In this study, we have developed Zika plasmid DNA vaccine that expressed codon-optimized 75 kDa of premembrane and envelope genes containing IgM signal sequence.

Methods

This constructed plasmid was transfected into mammalian cells and transgene expression was verified with western blot and fluorescence microscopy. Transfection studies revealed that recombinant protein was expressed and was actively secreted into cell supernatants. The immunogenicity of this DNA vaccine was also confirmed. Following vaccination of Balb/c mice via intradermal route by electroporation, all vaccinated animals developed premembrane and envelope-specific binding antibodies.

Conclusions

Our study indicates that DNA vaccine encoding codon-optimized premembrane and envelope with IgM signal sequence can induce immune responses, thus providing a useful approach to protect against Zika virus infection.

FEMS7-2850
Virology

NEW VARIANT OF RABBIT HAEMORRHAGIC DISEASE VIRUS (RHDVB) IN YOUNG RABBITS: CLINICAL AND PATHOLOGICAL CHARACTERIZATION

S. Pacho-jimenez[1], E. Dahdouh[1], A. Fernandez-manzano[2], A. Rodriguez-bertos[2], M. Suarez-rodriguez[1]
[1]Universidad Complutense De Madrid, Facultad De Veterinaria-sanidad Animal, Madrid, Spain
[2]Universidad Complutense De Madrid, Visavet, Madrid, Spain

Backgrounds

Rabbit Haemorrhagic Disease (RHD) is caused by infection with a rabbit calicivirus. It was first reported to affect adult rabbits and to result in very high morbidity and mortality rates. Symptoms of RHD include liver necrosis, pulmonary oedema and Disseminated Intravascular Coagulation (DIC), and leads to death within 24-48 hours. In 2010, a new distinct variant of the virus (RHDVb) was reported and was found to also infect young rabbits and to show symptoms similar to the classical variant.

Objectives

Since RHDVb has the potential of causing devastating outbreaks among young rabbits, our objective is to characterize the gross and histopathological alterations of two RHDVb isolates recovered from infected young rabbits in Spain.

Methods

Forty rabbits were infected with two different RHDVb isolates and necropsies were performed after death. Samples from the liver, lungs, spleen, kidneys, heart, lymph nodes, trachea, and brain were obtained for histological study.

Conclusions

Results and Discussion

Clinical and pathological findings revealed that one of the RHDVb strains induced a faster and more fatal disease in comparison with the second. Moreover, the lesions produced by this isolate were more extensive and intense. These preliminary outcomes showed marked differences among isolates of the same RHDVb where one displayed higher pathogenicity that the other.

Conclusions

In this study, we showed that two different isolates of RHDVb produced different clinical and pathological manifestation of RHD among young rabbits. Further investigations into the genetic differences between these isolates are necessary before determining the mechanisms that could explain this difference.

FEMS7-2957
Virology

ECTROMELIA VIRUS INDUCES TUBULIN CYTOSKELETON REARRANGEMENT IN MYELOID DENDRITIC CELLS, ACCOMPANIED BY LOSS OF MICROTUBULE ORGANIZING CENTER AND INCREASED A-TUBULIN ACETYLATION

L. Szulc-Dabrowska[1], M. Palusinski[1], J. Struzik[1], F.N. Toka[1,2]
[1]Warsaw University of Life Sciences,
Faculty of Veterinary Medicine- Department of Preclinical Sciences, Warsaw, Poland
[2]Ross University School of Veterinary Medicine, Department of Biomedical Sciences, Basseterre, St Kitts & Nevis

Backgrounds

Ectromelia virus (ECTV) belongs to the *Orthopoxvirus* genus of the *Poxviridae* family. It is a natural pathogen of mice that causes mousepox, a lethal disease of certain strains of mice. ECTV is a suitable model to study immunobiology of variola virus (VARV) and other orthopoxviruses, as well as pathogenesis of generalized viral infections. Orthopoxviruses exploit the host cell cytoskeleton for penetration, transport and release of progeny viral particles. So far, it is not known how orthopoxviruses influence the cytoskeleton of immune cells, especially dendritic cells (DCs), in which those viruses (with the exception of ECTV) undergo an abortive replication cycle.

Objectives

The aim was to investigate the effect of ECTV infection on the morphology of the tubulin cytoskeleton in myeloid DCs (mDCs).

Methods

mDCs were generated from murine bone marrow (BM) precursors using recombinant mouse granulocyte-macrophage colony stimulating factor (GM-CSF). BMDCs were infected with Moscow strain of ECTV and after 24 hours immunofluorescent staining and fluorescence microscopy analysis were performed.

Conclusions

The α-tubulin cytoskeleton in mDCs underwent dramatic rearrangement during ECTV infection. The microtubule network was disorganized, less intertwined and more “relaxed”. Decreased γ-tubulin staining indicated disappearance of the microtubule organizing centre (MTOC). Moreover, in infected cells increased acetylation of α-tubulin was observed. The elevated acetylation level of α-tubulin may lead to stabilization of microtubules, which are exploited by progeny ECTV virions for intracellular transport.

This work was supported by grants UMO-2011/03/B/NZ6/03856 and UMO-2012/05/D/NZ6/02916 from the National Science Center in Cracow, Poland, to LS-D.

FEMS7-2974
Virology

MYELOID DENDRITIC CELLS PRODUCTIVELY INFECTED WITH ECTROMELIA VIRUS HAVE IMPAIRED INNATE AND ADAPTIVE IMMUNE FUNCTIONS

L. Szulc-Dabrowska[1], J. Struzik[1], F.N. Toka[1,2], M. Bossowska-Nowicka[1], A. Ostrowska[3], Z. Nowak[4], M. Gierynska[1]
[1]Warsaw University of Life Sciences,
Faculty of Veterinary Medicine- Department of Preclinical Sciences, Warsaw, Poland
[2]Ross University School of Veterinary Medicine, Department of Biomedical Sciences, Basseterre, St Kitts & Nevis
[3]Warsaw University of Life Sciences, Analytical Center, Warsaw, Poland
[4]Warsaw University of Life Sciences,
Faculty of Animal Sciences- Department of Genetics and Animal Breeding, Warsaw, Poland

Backgrounds

Myeloid dendritic cells (mDCs) are key antigen presenting cells responsible for initiation of adaptive antiviral immune response. Some viruses employ sophisticated and host-specific strategies to control the immune cells that are important for induction of antiviral immune response. In particular, this can be observed in viruses that exhibit a narrow host range and have co-evolved with their natural host. A good example is the ectromelia virus (ECTV) of mice – an orthopoxvirus closely related to variola virus.

Objectives

The aim of this study was to investigate the impact of ECTV infection on immune functions of mDCs.

Methods

Bone marrow DCs (BMDCs) were prepared from BALB/c mice and infected with ECTV and additionally treated with LPS, with appropriate controls. After 24 h cells were used in experiments to determine cell phenotype, cytokine production, gene expression and signal transduction.

Conclusions

Infected mDCs showed inability to uptake and process antigen. mDCs did not reach a mature phenotype and were unable to secrete proinflammatory cytokines or induce T cell responses. Decreased cytokine/chemokine response resulted from impaired ligand-induced nuclear accumulation of NF-κB, IRF3 and IRF7 transcription factors and down-regulation of many genes involved in TLR, RLR and NLR signaling pathways. Moreover, ECTV-infected mDCs failed to stimulate proliferation of allogeneic $CD4^+$ T cells in mixed lymphocyte reaction. Overall, our results demonstrate a massive disruption of innate and adaptive immune functions in ECTV-infected mDCs, which compromises their ability to initiate downstream T-cell activation events.

The work was supported by grant No. UMO-2012/05/D/NZ6/02916 from the National Science Center, Cracow, Poland, to LS-D.

FEMS7-3201
Virology

ANALYZING THE INTERACTIONS BETWEEN THE HIV-1 GENOMIC RNA AND HOST PROTEINS THROUGH PROXIMITY LIGATION ASSAY

D. Toro Ascuy[1], B. Rojas-araya[1], A. Leyva[1], A. Dellarossa[1], F. Valiente-echeverria[1], R. Soto-rifo[1]
[1]Universidad de Chile, Virología, Santiago de Chile, Chile

Backgrounds

Human Immunodeficiency Virus type-1 (HIV-1) gene expression is highly complex and tightly regulated. Once the host RNA polymerase II recognizes the viral promoter it drives the synthesis of one single messenger RNA molecule, the 9-kb genomic RNA (gRNA), which first undergoes alternative splicing early during replication. Later on, the gRNA in its unspliced form is used as an mRNA for the synthesis of structural proteins and enzymes but also as the packaged genome. The molecular events that allow the interaction between the gRNA and the cellular machineries for nuclear export, translation or packaging have not yet been fully elucidated. The viral protein Rev has been identified as key viral component involved in nuclear export, translation and encapsidation of the unspliced transcript suggesting that Rev is an active component of different HIV-1 gRNA ribonucleoprotein complexes throughout viral replication.

Objectives

The aim of this study was to analyze the interactions between the HIV-1 genomic RNA and host proteins through proximity ligation assay (PLA).

Methods

We adapted the PLA, which is usually used to analyze protein/protein interactions, and characterized interactions between the HIV-1 gRNA and host proteins involved in nuclear export and translation initiation.

Conclusions

Our results validate the PLA as a simple and useful tool to study HIV-1 gRNA/protein interactions within cells. This protocol also serves to determine the subcellular locations where gRNA/protein interactions occur

FONDECYT Postdoctoral Grant 3160091 and FONDECYT Grant 1160176

FEMS7-2682
Virology

GENETIC AND LIFE-HISTORY TRAITS ASSOCIATED WITH THE DISTRIBUTION OF PROPHAGES IN BACTERIA

M. Touchon[1], *A. Bernheim*[1], *E. Rocha*[1]
[1]*Institut Pasteur - UMR3525, Microbial Evolutionary Genomics, Paris, France*

Backgrounds

Nearly half of the sequenced bacteria are lysogens and many of their prophages encode adaptive traits. Yet, the variables driving prophage distribution remain undetermined

Objectives

We identified 2246 prophages in complete bacterial genomes, using comparative genomic approaches, to study the genetic and life-history traits associated with lysogeny.

Methods

While optimal growth temperatures and average cell volumes were not associated with lysogeny, prophages were more frequent in pathogens and in bacteria with small minimal doubling times. Their frequency also increased with genome size, but only for genomes smaller than 6 Mb. The number of spacers in CRISPR-Cas systems and the frequency of type III systems were anticorrelated with prophage frequency, but lysogens were more likely to encode type I and type II systems. The minimal doubling time was the trait most correlated with lysogeny, followed by genome size and pathogenicity.

Conclusions

We propose that bacteria with highly variable growth rates often encounter lower opportunity costs for lysogeny relative to lysis. These results contribute to explain the paucity of temperate phages in certain bacterial clades and of bacterial lysogens in certain environments. They suggest that genetic and life-history traits affect the contributions of temperate phages to bacterial genomes.

FEMS7-0628
Virology

CRISPR-CAS PROVIDES PARTIAL IMMUNITY AGAINST A PHAGE THAT ENCODES AN ANTI-CRISPR PROTEIN

S. van Houte[1]
[1]*environment and sustainability institute, biosciences, Penryn, United Kingdom*

Backgrounds

The CRISPR-Cas adaptive immune system is widespread in bacteria and can provide effective protection against phage infections. In response, phages evolved anti-CRISPR genes that inhibit bacterial adaptive immunity. Phage DMS3vir+*acrF1* encodes AcrF1, which binds the Csy3 subunit of the Csy complex of *Pseudomonas aeruginosa* strain PA14 and causes loss of DNA binding activity of the complex.

Objectives

Here we aim to examine the evolutionary consequences of *acrF1* on CRISPR immunity.

Methods

Using experimental evolution and infection assays,

Conclusions

we show that CRISPR-Cas still provides partial immunity against anti-CRISPR phage. If infected at low MOI, CRISPR drives phage to lower frequencies, but at high MOI, the phage can increase in frequency. The results are consistent with a model that AcrF1 expression may be “too little, too late” during a first infection, but upon secondary infection phage faces a weakened immune response that can be successfully blocked by AcrF1.

FEMS7-2392
Virology

DYNAMIC BIOFILM ARCHITECTURE CONFERS INDIVIDUAL AND COLLECTIVE MECHANISMS OF PHAGE PROTECTION

L. Vidakovic[1], P.K. Singh[1], R. Hartmann[1], C.D. Nadell[1], K. Drescher[1,2]
[1]Max Planck Institute for Terrestrial Microbiology, Max Planck Research Group Bacterial Biofilms, Marburg, Germany
[2]Philipps University Marburg, Department of Physics, Marburg, Germany

Backgrounds

Bacteria often live in surface-attached communities, termed biofilms. Within these communities bacteria secrete an extracellular matrix that can protect cells from exogenous stress. In their natural environments, biofilms regularly encounter the presence of viral pathogens, termed bacteriophages.

Objectives

Our aim was to understand the mechanisms that determine phage-biofilm interactions and the infection dynamics.

Methods

In order to study phage-biofilm interactions we developed a method to visualize phage spread inside living *E. coli* biofilms. By insertion of *sfgfp* into the T7 phage genome, the conversion of susceptible to infected cells can be tracked spatiotemporally at the single-cell level using a recently-developed custom confocal microscope. Using a combination of bacterial genetics, molecular biology, and fluorescent reporters, we were able to understand key elements of phage-biofilm interactions.

Conclusions

Biofilm susceptibility to phage infection is dependent on the stage of biofilm development and the production of biofilm matrix. The removal of curli, a major component of the *E. coli* matrix, generated biofilms that were rapidly infected by phages, regardless of the age of the biofilms. Visualization of curli fibers within biofilms further demonstrated a dynamic matrix composition. The development of phage tolerance in biofilms was synchronous with the production of curli fibers. We further discovered that curli-dependent biofilm protection is achieved by two mechanisms: (1) curli fibers prevent phages from diffusing inside biofilms, and (2) curli fibers protect individual cells from phage infection. Our results show that a single component of the biofilm matrix can provide individual as well as collective protection against phage infection.

FEMS7-3189
Virology

CHARACTERIZATION OF ASFV A238L PROTEIN INTERACTION SITES WITH NF-KB

G. Vigara Astillero[1], *D. Pérez-Núñez*[1], *Y. Revilla*[1]
[1]*Centro de biología molecular Severo Ochoa, Virology and Microbiology, Madrid, Spain*

Backgrounds

African Swine Fever virus (ASFV), is a large DNA virus infecting monocytes and macrophages of domestic pigs and wild board, causing an acute and mortal disease. Within its encoded proteins, the protein A238L, previously defined in our lab, has a special interest as it's capable of causing immune suppression on the host by interacting with several cellular immunomodulators such as NF-kB and NF-AT, among others, putatively acting as substitute of cellular proteins like IkB during infection.

Objectives

The immunomodulatory capabilities exhibited by viral A238L protein are of interest for a biotechnological use, by finding new compounds which mimicry the function of the viral protein, as potential anti- inflammatory drugs. Thus, the main objective of our project is to characterize the interaction sites of the viral protein A238L with NF-kB immunomodulator, allowing us to design pharmacophores.

Methods

For this means, we have generated a model comparing IkB structure with A238L protein, identifying several common structures on both proteins. Based on these results, we have identified several residues putatively implicated on the interaction of A238L with NF-kB, based on its homology with IkB. Currently, we are generating several mutants based on this information using directed-mutagenic assays, on a pcDNA-A238L vector. Our plan is to use these mutant proteins on luciferase-reporter assays to assess the NF-kB promoter activation, together with interaction studies in vitro.

Conclusions

With these studies, in addition to designing new drugs for inflammatory diseases, we will contribute to the thorough knowledge about ASFV, against which there are no known therapies or vaccines.

FEMS7-0768
Virology

FIRST STEPS IN ELABORATION OF PHAGOTHERAPY AGAINST AEROMONAS SALMONICIDA

A.T. Vincent[1,2,3], V.E. Paquet[1,2,3], A. Bernatchez[1,2,3], D. Tremblay[1,4], S. Moineau[1,4], S.J. Charette[1,2,3]
[1]Université Laval, Microbiology, Quebec, Canada
[2]Université Laval,
Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Quebec, Canada
[3]Université Laval, Institut de biologie intégrative et des systèmes, Quebec, Canada
[4]Université Laval, Groupe de Recherche en Écologie Buccale GREB, Quebec, Canada

Backgrounds

Antibiotic resistance is a global concern that negatively impacts the aquaculture industry. The Gram-negative waterborne bacterium *Aeromonas salmonicida* subsp. *salmonicida* is the etiological agent of furunculosis, one major fish disease in farms raising salmonids, leading to significant economic losses. Studies reported that many strains of *A. salmonicida* are now resistant to antibiotics, making it difficult to treat infections. Recent reports suggest that virulent phages could be used in a context where antibiotherapy is less effective.

Objectives

The present study investigated the potential of lytic phages as biocontrol agents against *A. salmonicida*. To do this, phages infecting *A. salmonicida* were collected and investigated at both genomic and microbiological levels.

Methods

The genome (double-stranded DNA) of 12 phages specific to *A. salmonicida* was sequenced by Illumina MiSeq and *de novo* assembled. The genomes were then analyzed using bioinformatics to find the pangenome of the data set, allowing a clustering of the phages. Finally, the host range of the phages was tested against a panel of 65 *A. salmonicida* isolates from various geographical origins.

Conclusions

Our results indicate that at least 6 genomic groups of phages can infect *A. salmonicida* and that some of them have a broad host range. In fact, the 65 strains could be infected by at least one phage and a cocktail of phages could be designed to cover the set of *A. salmonicida* strains tested. The next step will be to test *in vivo* the capacity of infected fish to recover from furunculosis after a phage treatment.

FEMS7-0175
Bacterial lifestyles associated with olive trees and other woody plants

WHAT DO GENOME SEQUENCES OF XYLELLA FASTIDIOSA TELL US ON HOST ADAPTATION AND LIFESTYLE OF XYLELLA

M.A. Jacques[1], *N. Denancé*[1], *M. Briand*[1]
[1]*INRA, IRHS, Beaucouzé, France*

The extension of international trading and global changes affect the distribution of diseases posing new threats to food production and the preservation of some plant species in the environment. Thus an in-depth knowledge on how pathogens adapt to their environment is a prerequisite for further control and surveillance strategies. *Xylella fastidiosa* is one of the most dangerous plant-pathogenic bacterium that recently emerged in Europe. More than 350 plant species are listed as hosts of this bacterium. While *X. fastidiosa* is responsible for economically important diseases in some crops, ornamentals, and trees, in most host plant it does not cause any symptoms; these plants serving as unseen inoculum reservoir. However, some plant species fail to support *X. fastidiosa* growth in environmental conditions favorable to this bacterium. Moreover, *X. fastidiosa* is a genetically heterogeneous species with six subspecies and a number of clusters of strains that more or less group strains according to host plants and/or diseases. Finally, *X. fastidiosa* is prone to recombination and in several cases recombination was associated to host shift in field. These characteristics make *X. fastidiosa* a good model to look for genetic determinants responsible for host adaptation and lifestyle. We use typing methods, comparative genomics, and develop tools to identify sequences associated with specific traits. Analyses of core and accessory genomes of sets of strains differing by their taxonomic assignation to a subspecies, host and places of isolation highlighted key elements involved in host specificity that could also be used to develop novel identification tests.

FEMS7-3322
Bacterial lifestyles associated with olive trees and other woody plants

XYLELLA FASTIDIOSA, THE GLOBAL THREAT OF A VECTOR BORNE, PLANT PATHOGEN POLYPHAGOUS BACTERIUM

M.M. López[1], D. Olmo[2], A. Nieto[2], A. Monterde[1], M. Montes-Borrego[3], J. Peñalver[1], K. Ouertani[1], C. Morente[1], I. Navarro[1], M. Teresa Gorris[1], E. Marco-Noales[1], B.B. Landa[3]
[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Carretera Moncada-Náquera km 4,5, Moncada 46113, Valencia, Spain
[2]Laboratorio oficial de sanidad vegetal de las Islas Baleares. Govern Balear. Eusebi Estada 145. 070 09, Palma de Mallorca, Spain
[3]Instituto de Agricultura Sostenible (IAS-CSIC). Avda. Menéndez Pidal, 14004, Córdoba, Spain

Xylella fastidiosa is a fastidious plant pathogenic bacterium, xylem localised and vector transmitted, considered a quarantine organism in the European Union (EU). There are several subspecies described and their scientific and economic importance is due to unique characteristics: it has more than 350 plant hosts, different behaviors in them, causes severe symptoms and economic losses, has a long list of vectors and very difficult control. Originally discovered in America, X. fastidiosa subsp. pauca was reported on olive trees in southern Italy in 2013, where it is now responsible of the most severe disease never described in this crop. Comparative genomics suggests that it could have been introduced with plants from Costa Rica. After intensive surveys in the EU countries and analyses following the EPPO standard protocol, this pathogen was reported in France (Corsica and mainland) in 2015, in a greenhouse in Germany in 2016 and was also detected as widespread in Spain (Balearic islands) since 2016. Subspecies multiplex, fastidiosa and pauca, were found in these outbreaks. The detections of different subspecies of X. fastidiosa in few years in several EU countries, underline the presence of different genotypes and the risk of introducing additional genetic diversity. Recent information about X. fastidiosa biology and epidemiology suggests that the pathogen could have been present at least in Italy, France and Spain (and probably in other countries) for several years. This bacterium represents a threat not only for agricultural crops, but also for landscape trees and ornamentals, especially in Mediterranean countries.

FEMS7-0309
Bacterial lifestyles associated with olive trees and other woody plants

VIRULENCE AND ADAPTATION TO WOODY HOSTS IN THE BACTERIAL PHYTOPATHOGEN PSEUDOMONAS SAVASTANOI

E. Caballo-Ponce[1], I.M. Aragón[1], M.P. Castañeda-Ojeda[1], A. Pintado-Calvillo[1], A. Moreno-Pérez[1], A. Pérez-Baena[1], M. Martínez-Gil[1], J. Murillo[2], P. Rodríguez-Palenzuela[3], C. Ramos[1]
[1]Facultad de Ciencias- Biología- Universidad de Málaga, Biología Celular- Genética y Fisiología, Málaga, Spain
[2]ETS Ingenieros Agrónomos- Universidad Pública de Navarra, Laboratorio de Patología Vegetal, Pamplona, Spain
[3]Centro de Biotecnología y Genómica de Plantas CBGP. Universidad Politécnica de Madrid UPM-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA, Biotecnología, Pozuelo de Alarcón- Madrid, Spain

Bacteria belonging to the *Pseudomonas syringae* species complex, which includes *Pseudomonas savastanoi*, cause diseases in a broad variety of both woody and herbaceous plants. Although most research on the interactions established by this group of bacteria has focused on herbaceous plant pathogens, considerable efforts have been made during the last years to establish suitable models to study their interaction with woody plants. *P. savastanoi* produces aerial tumours on the host, causing one of the most important diseases of olive, but also infecting a plethora of plant species, including oleander, ash and broom plants. Moreover, *P. savastanoi* has recently been pointed out as an emergent pathogen of the ornamental species *Mandevilla sanderi* in several countries, including Spain (Caballo-Ponce *et al*., 2016). After the genome sequencing of the olive pathogen *P. savastanoi* pv. savastanoi NCPPB 3335 and the identification of novel virulence factors in this bacterium, our work has focused in the functional analysis of several of these virulence determinants, with special emphasis on specific factors associated with woody hosts. We have shown the translocation of eleven NCPPB 3335 type III secretion system effectors (T3E) into plant cells, some of which are associated to woody hosts (Castañeda-Ojeda *et al*., 2017). Other characterized virulence determinants include two enzymes involved in the metabolism of the bacterial second messenger c-di-GMP (Aragón *et al*., 2015a, 2015b) and several operons encoded in a genomic region of the NCPPB 3335 chromosome, named WHOP (from woody host and *Pseudomonas*), that is absent in *P. syringae* strains infecting herbaceous plants (Caballo-Ponce *et al*., 2017). Currently, we are studying the evolution of host specificity in *P. savastanoi* using the genome sequences of several strains isolated from different hosts.

References

Aragón *et al*., 2015a, Environ. Microbiol. 17: 4332–4351
Aragón *et al*., 2015b, Mol. Plant. Pathol. 16: 604-615
Caballo-Ponce *et al*., 2016, Plant Dis. 100: 2319
Caballo-Ponce *et al*., 2017, Mol. Plant Microbe. Interact. 30 (2) : 113-126
Castañeda-Ojeda *et al*., 2017, Mol. Plant Pathol. (early view : April 28th, 2016), doi: 10.1111/mpp.12420

Funding

Research supported by the Spanish Plan Nacional I+D+i grants AGL2014-53242-C2-1-R and AGL2014-53242-C2-2-R from the Ministerio de Economía y Competitividad (MINECO), co-financed by FEDER.

FEMS7-2598
Bacterial lifestyles associated with olive trees and other woody plants

BACTERIAL MULTISPECIES INTERACTIONS IN THE OLIVE-KNOT

V. Venturi[1], E. Caballo Ponce[2], M. Xianfa[1], G. Uzleac[1], C. Ramos[2]
[1]ICGEB, Bacteriology, Trieste, Italy
[2]Universidad de Malaga, Facultad de Ciencias, Malaga, Spain

It is believed that most bacteria live in constant association or in the vicinity of many different bacterial species. In addition, most bacteria are now believed to produce and respond to chemical signals in a cell-density dependent manner in a process known as quorum sensing (QS). QS results in a synchronous response of bacterial populations which confers them a form of multicellularity and enables them to adapt and survive to challenging environments. Most bacterial QS studies thus far have involved mono-species (in fact mono-strain) set up which are rather distant from what occurs in nature. It is our major interest to investigate chemical signaling in interspecies bacterial communities and the possible role of chemical signals in plant-bacteria interactions.

We are using beneficial bacterial endophytes which enter the plant via the rhizosphere to study interspecies interactions and dynamics of endophytic life style and multispecies community formation. This could lead to the use of endophytes as microbial products to improve plant health and sustainability in agriculture. In parallel we are studying bacterial interspecies signaling using a plant disease as a model. The olive knot disease caused by *Pseudomonas savastanoi* results in tumors/galls in olive trees; we have established that inside the tumors, together with the pathogen, other bacterial species interact and communicate with the pathogen resulting in mutual benefit and in a more aggressive disease.

FEMS7-0116
CRISPR as it is

CRISPR TECHNOLOGIES FOR BACTERIA

D. Bikard[1]
[1]*Institut Pasteur, Microbiology, Paris, France*

Over the past few years CRISPR systems have been derived into powerful tools to edit genomes, control gene expression, visualize nucleic acids in vivo, modify epigenetic marks, kill cells and more. Most of these tools rely on the RNA-guided nuclease known as Cas9 which can be easily reprogrammed to bind and cut almost any position in a genome. We focus on the application of these technologies to bacteria. We have recently investigated the properties of Cas9 as well as the catalytic dead variant known as dCas9 in *E. coli*. The introduction of double strand breaks by Cas9 at a specific genomic position leads to cell death as such beaks cannot be repaired through homologous recombination, the main DNA repair pathway in bacteria. We used this property as a selection tool in both *E. coli* and *S. pneumoniae*, and to develop sequence-specific antimicrobials against *S. aureus*. Work on the dCas9 protein has revealed how it can be used to precisely tune the expression level of several target genes independently and with low noise levels. Finally, the ability to knockdown gene expression with dCas9 can also be used in high-throughput screens to unravel gene function and interaction. All in all, CRISPR provide a fantastic toolkit to study and fight pathogenic bacteria.

FEMS7-0327
CRISPR as it is

MILESTONES IN CRISPR RESEARCH

F.J. Martinez Mojica[1], C. Almendros[1], N.M. Guzmán[1], B. Gómez-Tavira[1], J. García-Martínez[1], R. Maldonado[1]
[1]Univerisity of Alicante, Fisiología- Genética y Microbiología, San Vicente del Raspeig, Spain

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) were first seen in the bacterium *Escherichia coli* in 1987. In early 1990's, CRISPR arrays were also found in archaea where they appeared to be playing a relevant, yet enigmatic role. After verifying the widespread occurrence of CRISPR among prokaryotes, disparate *cas* (CRISPR-associated) genes could be identified and the diversity of CRISPR-Cas systems became evident. While diverse functions had been proposed for these systems since the discovery of the repeats, it was not until the origin of spacers (i.e., repeat-intervening sequences) was disclosed that the first hint in the right direction was provided: CRISPR are involved in interference against transmissible genetic elements targeted by the spacers. This observation led to the seminal demonstration that CRISPR-Cas enable prokaryotes to defend themselves against invading DNA. The mechanism responsible for achieving such adaptive immunity was conceived soon after the specific contribution of the main components of this system, repeats, spacers and certain Cas proteins, began to be understood. It was shown that CRISPR arrays are dynamic regions able to gain new spacers upon infection and generate single-spacer RNAs that through the repeat sequence guide Cas endonucleases against spacer-complementary sequences, resulting in target degradation. However, some CRISPR-Cas systems and stand-alone CRISPR regions or Cas proteins play roles not-related to immunity, relaying on regulation of gene expression or DNA repair activities among others, targeting DNA and/or RNA depending on the particular system. Thirty years after their discovery, many questions remain yet about these versatile, highly evolved repeat-based systems.

FEMS7-0049
CRISPR as it is

EVOLUTIONARY ECOLOGY OF CRISPR-CAS

E.R. Westra[1]
[1], *United Kingdom*

Bacteria have a range of sophisticated immune mechanisms to protect against virus infections, but it is unclear why all these different mechanisms evolved in the first place. Under laboratory conditions, bacteria typically evolve de novo virus resistance using either surface modification or CRISPR-Cas adaptive immune systems. In this talk I will discuss ecological factors that can tip the balance in the evolution of these two immune mechanisms and examine their distinct co-evolutionary implications.

FEMS7-1091
Epigenetics of bacterial infections

BACTERIAL INDIVIDUALITY AND COOPERATIVE VIRULENCE

M. Ackermann[1]
[1]*IBP, ETH Zurich and Eawag, Zurich, Switzerland*

A number of recent experiments has established that bacterial cells can act as individuals: genetically identical cells that reside in a homogeneous environment often vary in gene expression, activity and behaviour. This variation, which is known as phenotypic heterogeneity, can be biologically relevant: it can lead to the division of labor in clonal groups and also help these groups to survive external perturbations. I will focus on the biological significance of phenotypic heterogeneity in bacterial infections, using Salmonella Typhimurium as a model system. Clonal populations of S. Typhimurium differentiate into two subpopulations that differ in the expression of virulence genes. We find that this heterogeneity in virulence gene expression has important consequences: it leads to cooperative virulence that is required for effective infection of the host, and allows Salmonella to survive treatment with antibiotics. Intriguingly, there is an interplay between these two consequences, so that treatment with antibiotics can alter the infection dynamics and prolong the period of transmission. I will discuss whether some of these findings could represent general principles that apply to other pathogens and infections. Understanding how bacterial individuality impacts the dynamics of pathogen populations and the course of infections can potentially lead to new ways to control infections.

FEMS7-3306
Epigenetics of bacterial infections

NON MUTATIONAL RESISTANCE TO ANTIBACTERIAL AGENTS

J. Casadesus[1]
[1]*Facultad de Biología, Departamento de Genética, Sevilla, Spain*

Non mutational preadaptation to lethal selection

Josep Casadesús

Departamento de Genética, Universidad de Sevilla, Sevilla, Spain

When a bacterial population is subjected to lethal selection (e. g., encounter with a phage or exposure to an antibacterial agent), preexisting mutant cells survive. In certain cases, however, wild type cells can also survive. Non mutational preadaptation is made possible by the existence of phenotypic heterogeneity in isogenic populations of bacteria. If a physiological state that permits survival is propagated by a feedback loop, a progeny of adapted cells is formed. In certain cases, the feedback loop is a heritable DNA methylation pattern. In other cases, however, feedback loops can be formed without the involvement of DNA epigenetic marks. Three examples are as follows:

(i) Exposure of *Salmonella* to bile triggers the RpoS-dependent general stress response, which increases bile resistance. Because the *rpoS* promoter is noisy, certain *Salmonella* cells activate the general stress response in the absence of bile. High RpoS expression permits survival of certain cells in the presence of bile, and a positive feedback loop sustains or even amplifies the RpoS response, giving rise to a bile-resistant population.

(ii) Random variations in the expression or the activity of critical cell functions can confer adaptive resistance to certain antibiotics. In *Salmonella*, for instance, expression of porin genes is noisy, and reduced porin synthesis confers kanamycin resistance. Because transcription of porin decreases in the presence of kanamycin, a negative feedback loop propagates kanamycin resistance.

(iii) Nalidixic acid resistance requires mutation (e. g., in the QRDR region of *gyrA*). However, the level of nalidixic resistance conferred by a QRDR mutation can be further increased by active efflux mediated by the AcrAB-TolC pump. Cell-to-cell differences in efflux occur, and cells with high efflux show increased nalidixic acid resistance. Because nalidixic acid activates efflux, the response is propagated by a positive feedback loop.

FEMS7-2235
Epigenetics of bacterial infections

PHASE VARIATION IN BACTERIAL PATHOGENS

M. Van der Woude[1]
[1]*University of York, Centre of Immunology and Infection- HYMS and Biology, York, United Kingdom*

Phase variation is a common virulence strategy among bacterial pathogens. This heritable but reversible regulation frequently affects the cell surface and leads to single cell heterogeneity in a clonal population. One molecular mechanism is the result of epigenetic regulation that is dependent on changes in the DNA methylation pattern at specific Dam target sequences. Key features of this regulatory mechanism will be presented, which also illustrates why occurrence of this specific type of phase variation can remain undetected based on genome sequence analyses.

The O-antigen of the LPS is one of the defining features for a Salmonella serovar, and is used in diagnostics. We have identified families of O-antigen modifying genes in salmonella genomes and using our identified “phase variation signature sequence” show that the majority will be controlled by epigenetic phase variation. This alters the antigenicity, and thus likely is an immune evasion mechanism. However, our work shows that modifying the O-antigen composition has other important effects on the population. This work will be presented and illustrate how this epigenetic regulation in salmonella may impact on diagnostics, serovar evolution, and host-pathogen interactions. Our findings will be placed into the broader context of Dam and salmonella virulence, and on DNA methylation in bacterial pathogens in general.

FEMS7-2926
Fermented beverages: An interesting side of microbial abilities

IMPACT OF NUTRITIONAL AND TEMPARATURE STRESSES ON YEAST FERMENTATIONS

E. García-Rios[1], Y. Su[1], S. Muñiz[1], A. Querol[1], J.M. Guillamón[1]
[1]Institute of Agrochemistry and Food Technology IATA-CSIC, Food Biotechnology, Paterna, Spain

Our group is interested in understanding the mechanisms involved in adaptation that have shaped the yeast genome conferring properties of biotechnological interest. Different –omics techniques as well as evolutionary analysis are used to understand adaptation of yeasts of industrial interest to stresses. In this presentation, our insights gained in two of the most important environmental and nutritional stresses (low temperature and nitrogen availability) during wine fermentation will be shown. Low temperature alcoholic fermentations (10-15ºC) are becoming more frequent due to the winemaker's tendency to produce wines with more pronounced aromatic profile. However, this temperature is far from the optimum range for the main fermentative yeast *S. cerevisiae*, affecting both the yeast growth and fermentation rates. Nitrogen is an essential nutrient for yeast during wine fermentation. The deficiency of nitrogen is one of the major reasons of sluggish or stuck fermentation. Therefore, it is of great interest to characterize the nitrogen requirement of *S. cerevisiae* industrial strains. Curiously, our data reinforce the connection between both stresses. Low temperature firstly impacts on plasma membrane fluidity, affecting primarily to cellular nutrient uptake. Thus, low temperature and nutritional stress share many adaptative mechanisms in yeasts during industrial fermentations. Our approach has been to identify key physiological, metabolic and molecular mechanisms involved in the overcome of both stresses, and use this information for the design of rational genetic improvement strategies to obtain more robust and adapted yeast strains to grow during stressful industrial conditions.

FEMS7-3289
Fermented beverages: An interesting side of microbial abilities

MOTIVES AND MAPS TO ENJOY SPANISH FERMENTED FOODS AND BEVERAGES
H. Martín[1]
[1]*Universidad Complutense de Madrid, Microbiología II, Madrid, Spain*

MOTIVES AND MAPS TO ENJOY SPANISH FERMENTED FOODS AND BEVERAGES

Humberto Martín

Dpto. Microbiología II. Facultad de Farmacia. Universidad Complutense de Madrid and IRYCIS (Instituto Ramón y Cajal de Investigación Sanitaria)

Traditional fermented foods and beverages have been produced and consumed since the first civilizations. Their increased storage life, safety, pleasant flavors and effects on the well-being of the consumers were highly appreciated properties, becoming essential components of the human diet. Today, although the industrialization has reduced their number, there are still hundreds of fermented foods and beverages, and many of them represent a great cultural heritage in many countries. Microorganisms involved in fermentative processes transform meat, vegetables, dairy, legumes, cereals and fruits, often into stable microbial ecosystems. During this process, the involved microbes increase the bio-availability of nutrients, degrade toxic components and produce antimicrobial, antioxidant and bioactive compounds. All these abilities transform therefore the raw materials into potential functional foods.

A special category of the fermented products are represented by the alcoholic fermented beverages. Whereas the intake of high amounts of alcohol is well known to be harm for health, scientific reports consistently suggest that its moderate consumption by healthy adults seems to have beneficial effects, especially when considered as a part of the Mediterranean diet. A J-shaped relationship between alcohol consumption and mortality, with lower risk for moderate alcohol consumers than for abstainers or heavy drinkers is drawn by most epidemiological studies. In fact, a consensus scientific document on the effects on health of moderate beer consumption was published last year, showing evidence for no harm of moderate beer consumption for major chronic conditions and some benefit against cardiovascular disease.

Spain is a fascinating country for fermented products lovers, not only chesses, sausages or pickles, but especially for soft alcoholic beverages, including cider, wine or beer, with inexpensive and very high quality products. Some essential “maps” for enjoying them will be presented.

FEMS7-3280
Fermented beverages: An interesting side of microbial abilities

YEAST TO STUDY XANTHOHUMOL EFFECTS ON EUKARYOTIC CELLS

V. Mascaraque[1], J.M. Rodríguez-Peña[1], J. Arroyo[1], C. Nombela[1]
[1]Facultad de Farmacia. Universidad Complutense de Madrid UCM and Instituto Ramón y Cajal de Investigación Sanitaria IRYCIS,
Departamento de Microbiología II. Cátedra Extraordinaria de Bebidas Fermentadas, Madrid, Spain

Biomedical research carried out over the last years has reported xanthohumol (XN), the main prenylated flavonoid synthesized in hop cones, to be one of the polyphenols in beer responsible for many of the health benefits associated with its moderate consumption. Since XN is present only in those drinks and foods that contain hops, beer is almost the only dietary source of this flavonoid. XN possesses a wide range of pharmacological properties, including chemopreventive, anti-inflammatory, hypolipidemic, anti-infective and anti-oxidative bioactivities, giving this flavonoid promising potential in the prevention or treatment of different types of cancer, specific microbiological infections and several lifestyle-related diseases. Even though many health beneficial effects have been assigned to XN by studies in different mammalian cell lines, it is still unknown how XN mediates many of these effects and there is a lack of data about the regulatory network involved in those processes.

Saccharomyces cerevisiae is one of the most important yeast used for humans throughout history and not only in the food industry but also as a eukaryotic model microorganism with an invaluable role for the progress of biomedical sciences. For those reasons, the goal of the present work is to study how XN modulates cell physiology and the gene expression profile of *S. cerevisiae* cells in an effort to shed light on the mechanisms involved in XN response.

Our studies have revealed an association between the effect of XN and the metabolic stage of yeast cells. Cells in a high metabolic and energetic stage are more sensitive to XN than cells with a slower growth rate or in a non-proliferating stage. The analysis of the transcriptional profile and the transcription factors involved in XN response has disclosed a connection between some differently expressed genes and the physiological effects of XN observed on mammalian cells. Furthermore, an in depth study of the transcriptional response to XN has revealed the possibility that this flavonoid could hold some new potential anti-cancer activities.

FEMS7-3317
Fermented beverages: An interesting side of microbial abilities

DOMESTICATION OF BEER AND WINE YEASTS

J.P. Sampaio[1]
[1]*Universidade Nova Lisboa, Life Sciences, Caparica, Portugal*

Yeasts of the genus *Saccharomyces* are used since the dawn of civilization to ferment a myriad of foods and beverages that range from more familiar and globally dispersed products such as wine, beer and bread to less well-known and more locally-consumed beverages. Although it is usually assumed that *Saccharomyces* yeasts have underwent a domestication process equivalent to those already known for plant crops and livestock, the genomic underpinnings of yeast domestication remain unknown. Moreover, the natural history, ecology and distribution of *Saccharomyces* in truly natural habitats is poorly known, which hinders the study of the emergence of domesticated genotypes that likely derive from wild ancestors. Complete genome sequences made available by Next Generation Sequencing techniques, and phylogenetic and population analyses, allow an unprecedented fine scale resolution of population dynamics and organismic evolution. The combination of these approaches with detailed field ecology studies provides a powerful tool to study the evolutionary ecology, natural phylogeography and domestication history of *Saccharomyces*. Here, the most recent findings concerning the domestication trajectories of wine and beer yeasts will be discussed. The yeasts responsible for the fermentation of these two products, although belonging to the same species, appear to be quite distinct. For example, the nucleotide diversity of the main group of ale beer yeasts more than doubled that of wine yeasts, which might be a consequence of fundamental differences in the modes of beer and wine domestication.

FEMS7-0089
Marine microbiology

POLARIBACTER BLOOMS IN THE ARCTIC OCEAN: IS THERE A ROLE FOR PROTEORHODOPSIN?

C. Pedrós-Alió[1]
[1]*Centro Nacional de Biotecnologia ESQ2818002, Systems Biology, Cantoblanco. Madrid, Spain*

Backgrounds

The Arctic Ocean experiences major changes along the winter to summer transition. In previous studies we found that the planktonic microbial community changed composition around the time of ice breakup. Moreover, one Bacteroidetes genus, *Polaribacter*, usually dominated de Bacteroidetes assemblage. We analyzed the changes in abundance of this bacterium with time, reconstructed its gene content, and followed gene expression. In particular, we had seen a peak in expression of proteorhodopsin (PR) at ice breakup.

Objectives

We wanted to determine the potential importance of PR for the bloom.

Methods

We sampled surface water off Cambridge Bay (Canada) from early March to late June and obtained metagenomes and metatranscriptomes of three size fractions.

Conclusions

Bacteroidetes increased from 10% in March to 50% of the 16S rDNA reads in June, while chlorophyll a increased from 1 to 14 mg m^{-3}. *Polaribacter* became the dominant Bacteroidetes population making up 60% of the 16S rDNA reads. *Polaribacter* contigs assembled from the metagenomes had clusters of genes involved in extracellular polysaccharide degradation including groups of SusD-TonB genes (used for polymer degradation and transport of the monomers to the inside of the cell). These clusters showed synteny with those of *Polaribacter irgensii* (isolated from Antarctica) or from *Polaribacter* Hel-I-88 (isolated from Helgoland with relatively cold waters), but not with SAG MS024-2A or *Polaribacter* MED152 (isolated from warmer waters). This suggests that *Polaribacter* in cold waters prefer polysaccharides while those in temperate waters prefer proteins. Analysis of PRs is currently underway

FEMS7-1755
Marine microbiology

METAGENOMIC AND METATRANSCRIPTOMIC STUDIES OF THE MICROBIAL COMMUNITIES ASSOCIATED TO CULTURED CLAMS

J.L. Romalde[1]
[1]*CIBUS-Faculty of Biology. Universidade de Santiago de Compostela, Microbiology and Parasitology, Santiago de Compostela, Spain*

The bivalve culture sector, in particular the clam culture, is of great importance in Galicia (NW Spain). The overexploitation of the natural beds, together with other factors, have led to the import of seed and adult clams, with the subsequent risk of introduction of new pathologies (i.e. the appearance of the brown ring disease). During the last decade, different projects developed by our research group focused on the study of the culturable microbiota associated to clam populations allowed not only the identification of new bacterial genera and/or species, determining their possible effects on the clam growth, either negative (pathogenic potential) or beneficial (probiotic activity), but also to check the sanitary condition of the clam populations. The use of culture-independent techniques, namely next-generation sequencing, was addressed to get a comprehensive understanding of the bacterial assemblages within clam tissues, avoiding the limitations the culture-based procedures.
The studies included clam populations of two species, Manila clam (Ruditapes philippinarum) and carpet-shell clam (Ruditapes decussatus) from two localities in the Galician coast. Samplings were carried out in summer and winter to analyze any possible seasonal variation. Different organs, including mantle, gills, gonads, and hepatopancreas were analyzed also separately in order to determine differential tropisms of bacteria, as well as the ecological and functional role or the pathobiological key factors for the appearance of disease.
Overall, this metagenomic analysis revealed the presence of more than 15 bacterial phyla within the clam microbiome along with a significant number of unclassified bacteria, suggesting that these filter-feeding organisms are likely a rich source of bacterial repository that continues to be under examined.

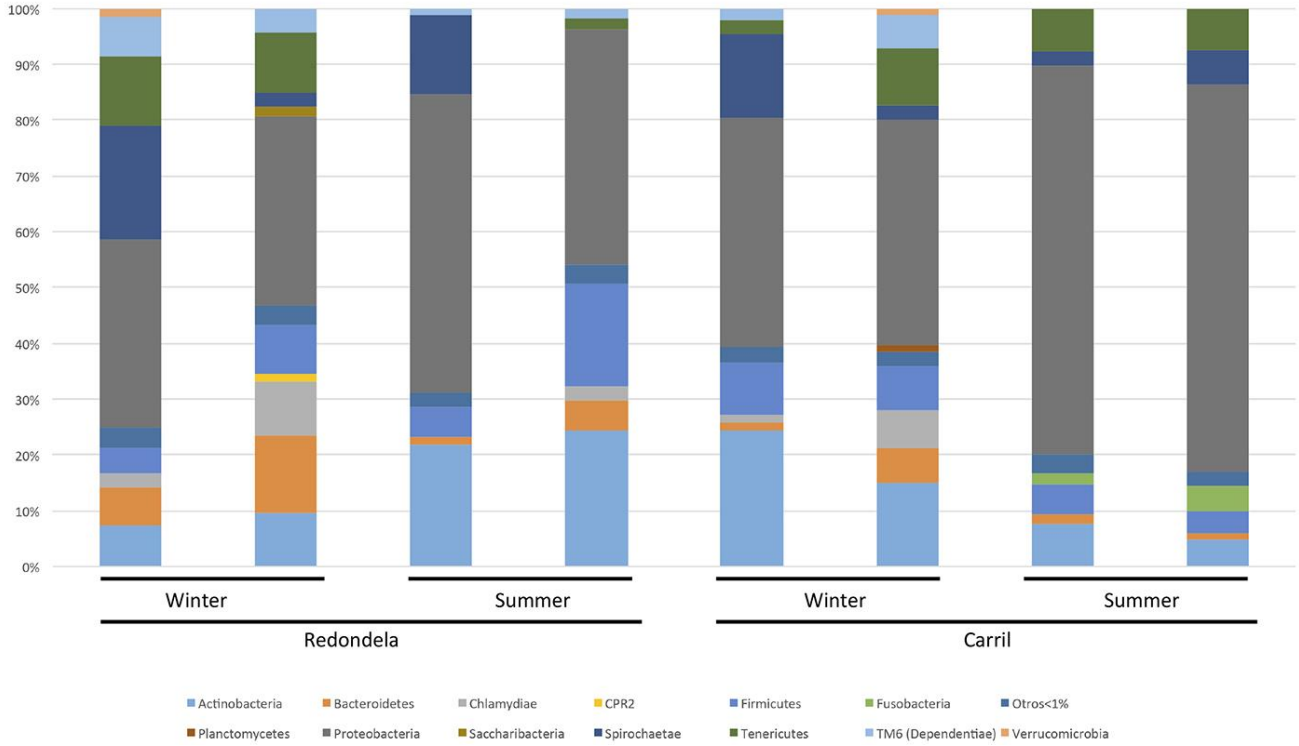

Figure 1.- Distribution of detected bacterial phyla in the clam samples obtained from two different locations in Galicia.

The highest number of Operational Taxonomic Units (OTUs) were observed in gonad and hepatopancreas tissues, which also rendered the highes species richness as calculated by Chao-1 index.

Although with relative differences associated to clam species or season, the most abundant taxa corresponded with Proteobacteria, Actinobacteria and Bacteriodetes (Fig. 1). It is noteworthy that most recovered bacterial groups by conventional culture-based techniques, namely Vibrio, Aliivibrio or Pseudoalteromonas among others, represented minor groups or were even not detected by metagenomic analysis. Conversely, some major groups in the 16S rRNA amplicons analysis were not detected with culture-based techniques. However, some of these organisms could be recovered using a dilution-to-extinction protocol, such as representatives of the genus Janthinobacterium.

The samples were also used for metatranscriptomic profiling aiming to reveal active metabolic pathways in the different clam organs and tissues. To obtain an overall profile of the gene functions throughout the different clam species, locations and seasons, coding sequences (CDSs) obtained from the samples were classified using COGs (Cluster of Orthologous Groups). An impressive variety of metabolic functions were found to be active in clam tissues, and their putative roles will be discussed.

This research was supported by grant AGL2013-42628R from the Ministerio de Economía y Competitividad (Spain).

FEMS7-0316
Marine microbiology

SPACE-TIME FRONTIERS OF MARINE VIRAL ECOLOGY

J.S. Weitz[1]
[1], *USA*

Viruses influence the fate of microbial cells and populations. Yet, there remain many obstacles to understand how virus-host interactions at the microscopic scale constrain and control macroscopic ecological patterns and ecosystem function. In this talk I highlight what theory and models can contribute to linking processes and patterns across scales. In doing so, I describe recent and ongoing efforts to characterize fluctuations in the relative abundances of marine viruses and their microbial hosts, infer virus-microbe infection networks in situ, and characterize the role and relevance of multiple infections.

FEMS7-0387
Role of microorganisms in the degradation of materials

FUNGAL DEGRADATION OF THERMAL-MODIFIED WOOD; ULTRASTRUCTURAL AND CHEMICAL ASPECTS

G. Daniel[1], J.S. Kim[1], J. Gao[1], M. Jebrane[1], N. Terziev[1]
[1]Swedish University of Agricultural Sciences, Forest Products, Uppsala, Sweden

Over the last decade the increasing awareness of environmental issues and legislation against the use of toxic wood protective chemicals has spurred the development of cost effective wood modification processes with an aim of not only reducing toxicity, but also retain and even increase the service life of wood. One of these processes termed “thermal modification (TM)” employs heat at elevated temperatures (160-220 ^{o}C) which causes changes in the wood structure whereby the dimensional stability can be increased and the durability and service life against fungal decay improved. One recent variation of the TM process known as thermo-vacuum (TV) employs a thermal wood process in which oxygen is removed by vacuum and heat transfer to the wood provided by convection in a so called “dry process”. Aspects on the physical and mechanical properties of TM wood as well as other macroscopical changes (e.g. density, color, swelling, mass loss, cracking) are quite well-known for a wide range of different TM treated wood species including both temperate and tropical soft- and hardwoods. However, despite the wide interest and great possibilities for using TM wood in-service, very little is known concerning modifications induced in the native wood material at ultra- and microstructural levels after treatments and how this effects decay by major fungal groups including white-, brown- and soft rot fungi.
Since all changes at macroscopic levels are derived from ultra/microstructural changes, it is likely that such knowledge can help to further understand and develop TM processes. In the present work we have studied the durability of a number of temperate soft- (fir, spruce) and hardwoods (beech, ash) treated by the TV process at temperatures ranging between 160-220 ^{o}C using a variety of white- (*Trametes versicolor*, *Pycnoporus sanguineus*, *Phlebia radiata*), brown- (*Postia placenta*, *Gloeophyllum trabeum*) and soft (*Phialophora mutabilis*, *P. malorum*, *Chaetomium globosum*) rot fungi using EN 113 or AWPA E10-08 durability tests. Aspects of wood modification at ultra- and microstructural levels were studied using a number of histo/cytochemical staining, immuno-antibody approaches using light-, fluorescence- and transmission electron microscopy (TEM).
Studies showed that improvement in decay resistance (i.e. according to EN-350-1) of TV wood which was optimal in the range 200-220 ^{o}C for the different rot types and fungal species used can be traced to modifications (e.g. polymer loss/redistribution) at the wood cell wall level in pectins (homogalacturonans, rhamnogalacturonan), hemicelluloses (xylan, glucomannan) and lignin (guaiacyl, syringyl). The much greater variability in cellular composition and chemistry (i.e. high cell wall hemicellulose composition) and range of different cell types with different chemistry in hardwoods compared with softwoods provides for the much greater difficulties for developing protection. In the softwoods and at high temperatures (i.e. > 200 ^{o}C), increased protection against decay was provided at the molecular level in the S2 layer by densification and lignin aggregation. TV modification only produced marked changes in decay morphology for soft rot in both hard- and softwoods which likely reflect modification of the non-cellulosic polymers and lignin. In the present talk, an overview will be given of the effects TM treatments have on the ultrastructure of wood cell walls and the implications this has for protection against major wood decay fungi.

FEMS7-1136
Role of microorganisms in the degradation of materials

MINERAL-MICROBIAL INTERACTIONS: GEOMICROBIOLOGY AND BIODEGRADATION

A. De los Ríos[1], *C. Ascaso*[1]
[1]*Museo Nacional de Ciencias Naturales-CSIC, Biogeoquímica y ecología microbiana, Madrid, Spain*

The biological and mineral components of terrestrial microbial ecosystems cannot be separately analyzed because of intimate mineral-microorganism relationships. Microorganisms colonizing rocks actively interact with the minerals of the colonized substrate but microorganisms within soil crusts and polar cryptogamic covers are also frequently associated with minerals. These relationships give rise to complex mineral-microorganism interactions, which result in physical and chemical alteration of the minerals and rocks. Such interactions are essential for initial pedogenesis processes and ecosystem functioning. Most microorganism-mineral interactions occur within an organo-mineral matrix composed mainly of a mixture of EPS and different autochthonous and allochthonous mineral constituents. The interactions established likely dependent on the three-dimensional organization of biotic and abiotic factors, and evolve over the time.

Microorganisms are the main form of terrestrial life in extreme environments, such as those of desert areas, and pioneers in the colonization of new habitats. Geomicrobiological interactions play an important role in this colonization because they help form protective microenvironments and microhabitats in which microorganisms survive and may even thrive.

Built stone is also colonized by microorganisms, but this time, the impacts of mineral-microorganisms interactions are non-beneficial because they induce non-desirable changes in the colonized lithic substrate (biodeterioration). Such detrimental microbial activity is conditioned by the physicochemical properties of the stone used in different constructions and the environmental conditions. Mechanical actions are the main force behind these alterations but, in most cases, chemical actions also play a role.

FEMS7-1792
Role of microorganisms in the degradation of materials

MICROBIAL COLONISATION AFFECTS THE EFFICIENCY OF PHOTOVOLTAIC PANELS

A. Gorbushina[1], P.M. Martin-Sanchez[1], J. Günster[2], W.J. Broughton[1], M. Porcar[3]
[1]Bundesanstalt für Materialforschung und –prüfung BAM, Department 4. Materials and Environment, Berlin, Germany
[2]Bundesanstalt für Materialforschung und –prüfung BAM, Division 5.4. Ceramic Processing and Biomaterials, Berlin, Germany
[3]Universitat de València - Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Biotechnology and Synthetic Biology, Paterna - Valencia, Spain

Solar Panels: Accessible, Ubiquitous and Highly Standardised Collectors of Sub-aerial Biofilms

Morphologically simple microbial biofilms have prevailed since life began on Earth. Biofilms form at the interfaces of solids with gases or liquids and have multiple effects on substrate and element cycles. In geobiological terms, the most interesting microbial communities are those that form on solids exposed to air (sub-aerial). Microbial colonisers of the atmosphere-lithosphere interface include algae, cyanobacteria, fungi as well as heterotrophic bacteria and they have colonised virtually every rock surface throughout the entire geological history of the Earth. In addition to sequestering carbon, sub-aerial biofilms (SABs) actively participate in rock weathering. Rock-inhabiting SABs are the primary settlers on lava following volcanic eruptions and on rocks following the retreat of glaciers. SABs especially dominate hostile environments in which growth of higher vegetation is restricted especially in deserts, polar- and alpine-regions. SABs are the primary colonisers of lithospheric (e.g. rocks) and anthropogenic substrates (buildings, monuments, solar panels, etc.). Life at the solid material/atmosphere interface influences and is affected by both the underlying substrate and the microclimate surrounding it. Although sub-aerial life is ubiquitous, how SABs develop and importantly degrade underlying substrates can only be clarified in well-controlled experiments that often involve simplified model systems. So far, biofilm development on solar panels has been studied using; (i) metagenomics; (ii) *in situ* microscopy; and (ii) classical microbiological methods that are both qualitative and quantitative. Here we suggest that solar panel biofilms are accessible and highly relevant objects to study microbial ecology, geobiology and biodeterioration.

FEMS7-0416
Role of microorganisms in the degradation of materials

MICROORGANISMS IN DETERIORATION OF HISTORICAL BUILDING MATERIALS

B. Gutarowska[1]
[1], *Poland*

Microbial colonization of historical building materials can lead to severe deterioration. Historical building materials are exposed to changing atmospheric condition, varying humidity, temperature, pH, light, chemical pollution, what stimulate microorganisms to growth and metabolic activity. Macroscopic symptoms of biodeterioration include visible surface defects, fiber splitting, pitting, microbial biofilms, discoloration, bulging plaster, peeling paint, moreover mechanical properties are changed, what is important for structural elements. Direction of biodeterioration is determined by chemical composition of materials, their porosity, water permeability and availability of nutrients. Mechanism of deterioration of inorganic building materials (brick, mortar, stone) caused by moulds, bacteria including cyanobacteria, algae, lichens, bryophytes involves the reaction between microbial metabolites e.g. organic and inorganic acids with components in building materials, what leads to increase solubility of components or formation of salts, which may be washed out from the surface resulting in reduced compressive strength. Organic materials (wood) are biodegradated by the action of extracellular enzymes of colonizing insects, filamentous and decay fungi, bacteria, what leads to the decomposition of cellulose and other components. Studies demonstrate that a combination of many new generation methods is suitable solution for analysis of historical building' biodeterioration including: molecular methods (construction of clone libraries, high-throughput sequencing) to assess VBNC microorganisms; metabolomics analysis of chemical compounds profiles what indicates mechanisms of biodeterioration (QTOF-HPLC-MS, MALDI), electron microscopy imaging technics what shows degradation of building materials (FESEM-EDX). Lecture will present examples of biodeterioration of famous historical buildings in Poland, Italy, Austria and other countries.

FEMS7-0761
SEM-SEIMC: Antimicrobial stewardship: from the lab to the patients

ROLE OF MICROBIOLOGY LABORATORY IN ANTIMICROBIAL STEWARDSHIP PROGRAMS

R. Canton Moreno[1]
[1], *Madrid, Spain*

Clinical microbiology laboratories and clinical microbiologist play a pivotal role in antimicrobial stewardship programs. IDSA (*Infectious Diseases Society of America*) and SEIMC (Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica) have established in their respective guidelines of antimicrobial stewardship programs that clinical microbiologist must be included as core members of stewardship teams. More recently this role has been recognized by the ECDC (European Center for Disease Prevention and Control). Main activities of clinical microbiology laboratory include (rapid) identification of pathogens, antimicrobial susceptibility testing, phenotypic detection and molecular characterization of antimicrobial resistance microorganisms and their corresponding resistance mechanisms, detection and characterization of outbreaks, surveillance cultures and timeline reporting of microbiological findings. Moreover, clinical laboratories should incorporate typing techniques (rep-PCR, PFGE, MLST or in the future whole genome sequencing) and offer statistical reports. Relevance of these activities have been reinforced with the increasing worldwide frequency of multi-drug resistant (MDR) organisms, particularly with carbapenemase producing organisms and new resistance threats such as colistin resistance due to *mcr-1* transferable gene. The implementation of surveillance programs (either with culture or with molecular methods) for the detection of colonized patient with MDR organisms as part of antimicrobial stewardship programs has demonstrated clear benefits to reduce antimicrobial use and infections due to MDR organisms. Moreover, the introduction of new techniques that also decrease reporting time of clinical microbiology laboratories, such as molecular or proteomic (MALDI TOF MS) ones, has also impact in antimicrobial use and antimicrobial resistance rates. Both World Health Organization and United Nations have recognized the antimicrobial resistance as a public health problem and encourage nations to implement actions to reduce it. These actions include access to (rapid) microbiological diagnosis and better antimicrobial use.

FEMS7-0102
SEM-SEIMC: Antimicrobial stewardship: from the lab to the patients

ANTIMICROBIAL STEWARDSHIP PROGRAMS: THE EUROPEAN PERSPECTIVE

C. Pulcini[1]
[1]*CHU de Nancy, Vandoeuvre-Lès-Nancy, France*

This presentation will provide an overview of European antimicrobial stewardship initiatives, highlighting some innovative experiences and presenting some 'hot topics' in the field. Special attention will be paid to the role of microbiologists in antimicrobial stewardship programmes.

FEMS7-1345
SEM-SEV: Viral pathogenesis mediated by small RNAs and RNA silencing supressors

ROLE OF SMALL-RNAS IN VIROID PATHOGENESIS

F. Di Serio[1]
[1]*Institute for Sustainable Plant Protection CNR, National Research Council of Italy, Bari, Italy*

Viroids are tiny, circular, naked and infectious RNAs that replicate in the nuclei (family *Pospiviroidae*) or chloroplasts (family *Avsunviroidae*) and then invade systemically their host plants, which may react developing severe diseases. Viroid RNAs are targeted by Dicer-like (DCL) ribonucleases generating viroid-derived small RNAs (vd-sRNAs) of 21-24 nt that are loaded into Argonaute (AGO) proteins, the major effectors of RNA silencing. The biological roles of vd-sRNAs have been at least partially unveiled, with data supporting their involvement in anti-viroid defense and pathogenesis. In this respect, it has been shown that symptoms induced by a chloroplast-replicating viroid may be elicited by vd-sRNAs that, resembling structurally and functionally microRNAs, down-regulate the expression of a host gene and trigger pathogenesis via a post-transcriptional RNA silencing-based mechanism. A similar pathogenic mechanism has been proposed for several nuclear-replicating viroids, but the evidence seems less solid. Multidisciplinary approaches, mainly based on genome-wide approaches (degradome sequencing, deep sequencing of vd-sRNAs and transcriptome analyses) should help to clarify whether vd-sRNAs are actually involved in the pathogenesis elicited by nuclear-replicating viroids or alternative molecular mechanisms must be considered.

FEMS7-0574
SEM-SEV: Viral pathogenesis mediated by small RNAs and RNA silencing supressors

SEVERE OUTCOME OF INFLUENZA VIRUS-INFECTED PATIENTS CORRELATES WITH LOW PRODUCTION OF DEFECTIVE GENOMES

J. Vasilijevic[1], J.C. Oliveros[2], A. Rodriguez-Frandsen[3], S. Rey[4], I. Barba[5], G. Rodriguez[6], M. Perez-Ruiz[7], F. Pozo- I.Casas[8], A. Nieto[1], A. Falcon[9]
[1]National Centre for Biotechnology CNB-CSIC, Molecular and Cellular Biology, Madrid, Spain
[2]National Centre for Biotechnology CNB-CSIC, Bioinformatics for Genomics and Proteomics, Madrid, Spain
[3]Sanford-Burnham-Prebys Medical Discovery Institute, Infectious and Inflammatory Disease Center, La Jolla, USA
[4]University Hospital of Vigo, Microbiology, Vigo, Spain
[5]Complejo Hospitalario de Ciudad Real, Microbiology, Ciudad Real, Spain
[6]University Hospital San Pedro de Alcantara-, Microbiology, Cáceres, Spain
[7]Hospital Virgen de las Nieves, Microbiology, Granada, Spain
[8]National Centre of Microbiology- Institute of Health ISCIII, National Influenza Center, Madrid, Spain
[9]National Centre for Biotechnology CNB-CSIC. Network CIBER of Respiratory Disesases CIBERES, Molecular and Cellular Biology, Madrid, Spain

Severe outcome in influenza virus infected patients is associated with reduced accumulation of defective viral genomes

Influenza A virus (IAV) infection can be severe or even lethal in toddlers, the elderly and patients with certain medical conditions. Infection of apparently healthy individuals nonetheless accounts for many severe disease cases and deaths, suggesting that viruses with increased pathogenicity co-circulate with pandemic or epidemic viruses. Looking for potential virulence factors, we have identified a viral genetic determinant that contributes to infection outcome. A polymerase mutation identified in a fatal IAV case, when introduced into two different recombinant virus backbones, led to reduced defective viral genomes (DGs) production and increased pathogenesis in mice. These data provide genetic support for the association of pathogenicity and low DGs accumulation induced by mutations present in pathogenic viruses circulating in humans. Testing this association, we performed a genomic analysis of viruses isolated from a cohort of previously healthy individuals who suffered highly severe IAV infection requiring admission to Intensive Care Unit, and patients with fatal outcome who additionally showed underlying medical conditions. These viruses were compared with those isolated from a cohort of mild IAV patients. Viruses from highly severe/fatal outcome patients showed significantly fewer DGs accumulation than control viruses, suggesting that low DGs abundance constitutes a new virulence viral pathogenic marker in humans, regardless of the mutations responsible.

FEMS7-2368
SEM-SEV: Viral pathogenesis mediated by small RNAs and RNA silencing supressors

CONTRIBUTION OF RNA SILENCING SUPPRESSORS TO PATHOGENICITY IN POTYVIRAL INFECTIONS

B. Rodamilans[1], A. Gallo[1], A. Mingot[2], D. San León[1], A. Valli[1], J.J. Lopez-Moya[2], J.A. Garcia[1]
[1]Centro Nacional de Biotecnología, Genetica Molecular de Plantas, Madrid, Spain
[2]Centre for Research in Agricultural Genomics CSIC-IRTA-UAB-UB,
Program of Plant Responses to Stress, Barcelona, Spain

FUNCTIONAL DIVERSITY AND PARTIAL REDUNDANCY OF POTYVIRAL RNA SILENCING SUPPRESSORS

The concept of RNA silencing refers to a complex ensemble of regulatory mechanisms controlling gene expression in most eukaryotic organisms. Plants use this system as a major antiviral defense, forcing plant viruses to counteract it by developing RNA silencing suppressors (RSSs). Viral RSSs are very diverse and usually assume additional functions. That is the case of HCPro, the well-characterized RSS from members of the genus *Potyviru*s (family *Potyviridae*), which is also required for aphid transmission. However, members of other genera of this family express divergent HCPros lacking RNA silencing suppression, and surrogate this activity to other proteins also encoded by sequences at the 5'-terminal region of the viral genome. We have demonstrated that the transframe product P1N-PISPO from the potyvirus *Sweet potato feathery mottle virus* (SPFMV) shows weak silencing suppression activity in an agroinfiltration system. In agreement with this fact, P1N-PISPO was able to replace HCPro, although with very low efficiency, in the infection of the potyvirus *Plum pox virus*. In contrast, SPFMV HCPro did not appreciably suppressed RNA silencing in the agroinfiltration system, but supported the infection of a PPV variant lacking its native HCPro. Recently, we reported that a novel RSS-unrelated activity of PPV HCPro prevents CP degradation by forming stable virus particles. We have now demonstrated that this activity depends on virus replication and can be provided, at least partially, by SPFMV HCPro. Together, our results highlight the functional diversity and partial redundancy of RSSs supporting potyviral infections.

FEMS7-0268
SEM-SEV: Viral pathogenesis mediated by small RNAs and RNA silencing supressors

DEFECTIVE VIRAL GENOMES AS DETERMINANTS OF PARAMYXOVIRUS PATHOGENESIS

C. Lopez[1]
[1], *Philadelphia, USA*

The innate immune response controls viral replication or kill infected cells through the production of effector molecules, such as type I IFNs and TNF. Yet, viruses have evolved strategies to avoid elimination that allow them to persist in circulation. Defective viral genomes (DVGs) generated during the replication of most viruses have long been considered a viral product with evolutionary advantages in facilitating virus persistence. However, recent evidence demonstrates a contrasting critical role for DVGs in promoting strong induction of anti-viral immunity during infections with a number of RNA viruses. Why and how DVGs and host interact to achieve these two divergent and critical functions is poorly understood. Using RNA-FISH to probe DVGs and full-length (FL) viral genome at a single cell level during paramyxovirus infections, we identified a subset of infected cells that is dominated by DVGs (DVG-high) and is less prone to viral induced apoptosis than cell dominated by FL genomes. Transcriptional profiling revealed that DVG-high cells actively engage a strong TNF pro-survival program. Remarkably, TNF secreted in response to MAVS signaling during viral infection dictates the apoptosis of FL-high cells while supporting the survival of DVG-high cells. The MAVS/TNF axis leads to up-regulation of TRAF1/ TNFRII signaling in DVG-high cells extending their life span enough to promote the generation of persistent infections. Thus, our data suggest that DVGs are a pivotal component of the paramyxovirus-host interaction that determines the subsistence of virus and host by limiting acute virus replication while protecting a subset of infected cells from apoptosis thereby facilitating virus persistence.

FEMS7-0199
Structural nanomicrobiology

NANOSCALE APPROACHES TO A SYNTHETIC BACTERIAL AMYLOIDOSIS

R. Giraldo[1]
[1], *Spain*

Backgrounds

Protein amyloids arise from the conformational conversion and assembly of a soluble protein into fibrils with a crossed β-sheet backbone, leading to devastating proteinopathies. The WH1 domain of the bacterial protein RepA assembles into amyloid fibres *in vitro*.

Objectives

We aim to unravel amyloid structure, assembly and toxicity at the interface across Microbiology and Nanoscience.

Methods

Electron and atomic force microscopies, surface-enhanced Raman spectroscopy, microfluidics, bacterial genetics, protein engineering.

Conclusions

RepA-WH1 fibres are bundles of intertwined tubular protofilaments made of distorted protein monomers. Fibres can be nucleated by gold nanorods functionalized with the protein and assembly monitored as an increase in β-sheet. RepA-WH1 causes in *E. coli* a *vertically* transmissible amyloid proteinopathy. Bacterial lineages maintain two strains of RepA-WH1 amyloids and the DnaK chaperone modulates phase transitions between both. Oligomeric amyloidogenic precursors form at the bacterial nucleoid. RepA-WH1 builds pores through model membranes. RepA-WH1 is a minimal, synthetic bacterial model of an amyloid disease (reviewed in: *Prion* 10:41-9, 2016).

FEMS7-0333
Structural nanomicrobiology

HALF FERRITIN, HALF VIRUS: BACTERIAL SUBVERSION OF A BACTERIOPHAGE CAPSID AS AN IRON MEGASTORE

D. He[1], C. Piergentili[2], J. Ross[3], C.L. Mackay[4], K. Gallagher[3], K. Waldron[5], D. Clarke[3], J. Marles-Wright[2]
[1]Edinburgh University, Institute of Quantitative Biology- Biochemistry- and Biotechnology, Edinburgh, United Kingdom
[2]Newcastle University, School of Biology, Newcastle upon Tyne, United Kingdom
[3]Edinburgh University, School of Chemistry, Edinburgh, United Kingdom
[4]The University of Edinburgh, School of Chemistry, Edinburgh, United Kingdom
[5]Newcastle University, Institute of Cell and Molecular Biosciences, Newcastle upon Tyne, United Kingdom

Encapsulin nanocompartments are intracellular protein cages that are structurally related to the capsids of the HK97 bacteriophage and are distributed across many bacterial and archaeal species. Encapsulins sequester a number of different enzymes within their lumen through specific interactions with short localisation peptides on their cargo proteins. These cargoes include the well-characterised dye-dependent peroxidases, a new class of decameric ferritin, and a number of recently identified proteins with unknown functions. In the case of the dye-dependent peroxidases, the encapsulin cage protects the cell from oxidative damage resulting from the peroxide-radical mechanism these proteins employ to cleave carbon-carbon bonds in their substrates. Using X-ray crystallography in concert with structural Mass spectrometry, we have determined the structure of the decameric ferritin from *Rhodospirillum rubrum* and show that it possesses the ferroxidase activity found in other members of the ferritin family, but due to its open annular structure it is unable to directly sequester iron. We show that the encapsulin shell associated with these ferritins acts as a cage to store the ferric iron as ferrihydrite minerals. The encapsulin cages are between 25 and 35 nm in diameter, and have more interior space than the 8 nm ferritin nanocages; they are consequently able to store an order of magnitude more iron than classical ferritins. Our recent results on these decameric ferritins have identified the key residues responsible for both their multimerisation and ferroxidase activity.

FEMS7-0403
Structural nanomicrobiology

CRYSTALLOGRAPHIC STRUCTURES, RECEPTOR-BINDING AND APPLICATIONS OF VIRUS FIBRE PROTEINS

M.J. Van Raaij[1]
[1]*Centro Nacional de Biotecnología CNB-CSIC, Department of Macromolecular Structure, Madrid, Spain*

Both adenoviruses and bacteriophages use fibre proteins to recognize their host cells.Bacteriophages are complicated onetime nanomachines that transfer their genomic material into susceptible host bacteria. They have specialized proteins for initial, reversible, host cell wall recognition. Once a suitable host is found, the phage commits to infection by irreversible attachment via a secondary receptor interaction.
We have solved the detailed structures of several phage receptorbinding proteins and have shown them to be mainly betastructured, but structurally highly diverse and containing several new protein folds. Structures of the receptor-binding proteins of the coliphages T4, T5 and T7, of the Salmonella phage epsilon15 and of the Staphylococcus phages S241 and K will be shown.
Adenoviruses fibre proteins also serve as primary host cell recognition proteins and we have recently determined structures of the first atadenovirus and siadenovirus fibre head domains. We also discovered that the atadenovirus LH3 capsid protein contains a bacteriophage tailspike fold.
Ongoing structural, mutational and binding analysis of virus receptor-binding proteins with receptors and receptor analogues will be discussed. Bacteriophage receptor-recognizing proteins may be used for bacterial detection, while modification by natural or experimental mutation of bacteriophage receptorbinding domains may allow retargeting of phages to alternative host bacteria. Their shape and stability may also allow their use in nanotechnological applications.

FEMS7-0258
The pangenome of prokaryotic populations

IN-PATIENT EVOLUTION OF PSEUDOMONAS AERUGINOSA POPULATIONS IN HUMAN LUNGS

H.K. Johansen[1], J. Haagensen[2], R. Marvig[3], S. Molin[2]
[1]Rigshospitalet, Department of Clinical Microbiology, Copenhagen Ø, Denmark
[2]Technical University of Denmark, The Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark
[3]Rigshospitalet, Center for Genomic Medicine, Copenhagen Ø, Denmark

IN-PATIENT EVOLUTION OF *PSEUDOMONAS AERUGINOSA* POPULATIONS IN HUMAN LUNGS

Objective: Persistent airway infections with *Pseudomonas aeruginosa* are responsible for the gradually decreasing lung function in cystic fibrosis (CF) patients. The aim of our research is to identify which properties are involved in persistence of *P. aeruginosa* (PA) in the lungs of young CF patients.

Methods: Clinical isolates of PA have been collected for 10 years to cover the earliest infection stages. We have whole genome sequenced more than 600 PA isolates from 41 CF patients. From these sequences clone types were identified, and selected phenotypes, eg. antibiotic susceptibility, adhesion (biofilm) and growth rates were determined.

Results: Almost 75% of all CF patients were infected with the same clone-type during the entire collection period, despite intensive antibiotic therapy. Persistence was not associated with antibiotic resistance. We detected 23 tobramycin resistant isolates out of 379 (6%) isolates and 17 piperacillin + tazobactam resistant isolates out of 403 (4%) isolates. More than 20% of all isolates displayed reduced growth rates, and significantly more isolates had lower adherence compared to increased adherence (biofilm) ($p<0.0003$).

Conclusions: Most CF patients acquire persistent infections from their first colonization by PA, suggesting that current clinical definitions of colonization and chronic infection need to be revised. Persistence of PA cannot be explained phenotypically and genetically because antibiotic resistance is only observed in scattered early PA isolates, and the increased generation time is not associated with any clear pattern of antibiotic resistance. The reduced or unaffected biofilm formation over time suggests that persistence is not derived from biofilm development.

FEMS7-0070
The pangenome of prokaryotic populations

PROKARYOTIC VIRUSES, PANGENOMES AND EVOLUTIONARY SELECTION UNITS
F. Rodriguez-Valera[1]
[1], *Spain*

The genomic diversity present in concurrent individuals in a given bacterial population is enormous. Cells (or clonal lineages) contain different alleles of the genes in the core genome that vary typically ca. 5% in nucleotide identity but can have much higher local diversity. However, where these concurrent lineages display more divergence is in the flexible genome. Part of it is dispersed throughout the core in small synteny breaks, but about 50% of the flexible genome is concentrated in flexible genomic islands (fGIs). They can be classified in (at least) two different kinds. Additive fGIs like integrons vary by accretion of gene cassettes that code for biotic and abiotic niche interaction such as antibiotic resistance and production, metabolism and transport of specific substrates or cell regulation and chemotaxis. Additive fGIs vary very rapidly in evolutionary time scales through site directed recombination. Replacement fGIs code for outermost exposed polysaccharides (glycotypes) such as the O-chain or exopolysaccharides, they vary more parsimoniously by doble cross-over homologous recombination . This mechanism leads to complete exchange of the island by another present in a separate lineage. We propose that the presence of several glycotypes coexisting in the population is the key to the long range survival and stability of complex clone consortia by a negative density-dependent selection mechanism based on bacteriophages that recognise only some specific glycotypes. Furthermore, we would like to propose that a complex population made up of multiple clonal lineages and their cognate phages represent a single group selection unit.

FEMS7-0218
Yeast as eukaryotic models and cell factories

THE YEAST CHRONOLOGICAL AGING MODEL: PROTEOTOXIC STRESS AND AGE-RELATED DYSFUNCTIONS

P. Ludovico[1]
[1]*School of Medicine, Life and Health Sciences Research Institute ICVS, Braga, Portugal*

Background

Aging is a complex and multi-factorial process that results in the progressive accumulation of molecular alterations and disruption of different cellular functions. Genome instability, nutrient sensing, mitochondria dysfunction and loss of proteostasis are some of the proposed aging hallmarks, nevertheless the relationships between these phenomena have been largely unexplored. Yeast provides a simple and powerful model for cellular aging research particularly for the understanding of the relationships between aging hallmarks.

Objectives

In this presentation, the yeast chronological aging model will be explored and several examples of new insights produced in this cellular eukaryotic model will be discussed.

Methods

Studies on the yeast chronological aging model have shown that elevated levels of hydrogen peroxide, generated during caloric restriction, a manipulation that extends life span and retards age-related phenotypes in a variety of species, reduces the accumulation of superoxide anions. On the other hand, the use of yeast aged cells expressing the Parkinson's disease related protein, alpha-synuclein, provided new findings on loss of proteostasis during aging. Alpha-synuclein is a naturally prone to misfold protein impacting on protein quality control system such as autophagy. We found that increased autophagy and mitophagy observed in yeast cells expressing alpha-synuclein accelerates chronological aging. The regulation of autophagy and its crosstalk with genomic instability will be discussed.

Conclusions

Overall, the data to be presented gives new insights on cellular mechanisms of aging. "*All the models are wrong but some are useful*" and this is the case of the yeast aging model.

FEMS7-2221
Yeast as eukaryotic models and cell factories

YEAST AS A MODEL FOR UNDERSTANDING CELL SIGNALING-ASSOCIATED PATHOLOGIES

I. Rodríguez-Escudero[1], T. Fernández-Acero[1], M.D. Oliver[1], I. Bravo[1], R. Pulido[2], V.J. Cid[1], M. Molina[1]
[1]Universidad Complutense, Dpt. Microbiología II. Facultad de Farmacia, Madrid, Spain
[2]BioCruces Health Research Institute, Cancer Research, Barakaldo, Spain

Given the conservation of signaling mechanisms and cellular processes along the evolutionary scale, the yeast *Saccharomyces cerevisiae*, a readily manageable unicellular eukaryotic organism, represents an excellent tool for the functional study of proteins of complex organisms that, upon heterologous expression, are able to couple or interfere with yeast signaling or growth. This approach, often referred as 'humanizing yeast', also allows the development of yeast-based bioassays adaptable to high throughput screening (HTS), for identifying specific inhibitors of human proteins involved in disease. One successful example carried out by our group is the reconstitution in yeast of the oncogenic pathway involving PI3K (phosphatidylinositol 3-kinase), PTEN (phosphatidylinositol 3-phosphatase) and protein kinase Akt.

The aim of this talk is to analyse the scope as well as the pros and cons of using yeast as a model for studying cell signaling- associated diseases and drug discovery, exemplified by the PI3K pathway. Functional analyses on yeast cells expressing human proteins involved in PI3K signaling have been performed by using growth assays, directed and random mutagenesis, cell and molecular biology techniques and functional genomics (transcriptomics, genomic mutant collections, etc). By this means, the PI3K humanized model yeast allowed us to identify oncogenic mutations in catalytic and regulatory PI3K subunits, PTEN and Akt, as well as PI3K inhibitors to be developed for anticancer therapy. Functional studies on the putatively therapeutic long version of PTEN have been also feasible in this model. At the same time, the system has proven to be useful to further understanding yeast physiology, particularly lipid signaling.

FEMS7-0493
Yeast as eukaryotic models and cell factories

UNDERSTANDING THE MECHANISM OF SACCHAROMYCES SPECIES ADAPTATION TO WINE FERMENTATIONS

A. Querol[1], E. Navarro-Tapia[2], R. Pérez-Torrado[3]
[1], Spain
[2]Institute of Agrochemistry and Food Technology IATA-CSIC, Food Biotechnology, Paterna, Spain
[3]Institute of Agrochemistry and Food Technology IATA-CSIC, Food Biotechnology, 46980, Spain

In wine fermentation, high ethanol concentrations is one of the main metabolites responsible of problematic fermentations. The adaptation to high ethanol is also very important in the cases of sparkling and Sherry wines. Although many efforts have been made, mechanisms of ethanol tolerance are not fully understood in *Saccharomyces cerevisiae*. This work studied two *S. cerevisiae* strains, CECT10094 and Temohaya-MI26, isolated from flor wine and agave fermentation (a traditional fermentation from Mexico) respectively, which differ in ethanol tolerance, in order to understand the molecular mechanisms underlying the ethanol stress response and the reasons for different ethanol tolerance.

The transcriptome was analyzed after ethanol stress and, among others, an increased activation of genes related with the unfolded protein response (UPR) and its transcription factor, Hac1p, was observed in the tolerant strain CECT10094.

Although our data and others authors ensure that the UPR is triggered by the unfolded proteins generated by ethanol in the cell, the mechanism of activation not being known. In this study we have investigated the activation of the UPR response in yeast *S. cerevisiae* by ethanol stress and we want to know if really the ethanol produces unfolded proteins and if this is the mechanism of activation of UPR after an ethanol stress. According these data, we can conclude that, although UPR activation was observed, unfolded proteins do not accumulate in the ER under 8% (v/v) ethanol stress. We also ruled out inositol depletion as an alternative mechanism to activate the UPR under ethanol stress.

FEMS7-3284
Yeast as eukaryotic models and cell factories

YEAST PHYSIOLOGICAL TOXICOGENOMICS: INSTRUMENTAL TO GUIDE THE DEVELOPMENT OF ROBUST CELL FACTORIES

I. Sa-Correia[1]
[1]*iBB-Institute for Bioengineering and Biosciences- Instituto Superior Técnico, Bioengineering, Lisbon, Portugal*

Background: The improvement of the capacity of industrially relevant yeasts to tolerate toxic substrates or products, combined with operating conditions that do not allow maximum stress tolerance, is an important challenge of modern Biotechnology. Yeast physiological toxicogenomics provides a holistic assessment of the complex adaptive responses to chemical stresses and the identification of tolerance or susceptibility determinants, at a genome-wide scale. [1] This knowledge is instrumental to guide synthetic pathway engineering and other practices for increased cell robustness manipulation either for the sustainable production of fuels and chemicals or for the control of food and beverage spoilage yeasts.

Objectives: This talk will focus on our current knowledge on the physiological genomics of the adaptive response and tolerance to relevant stresses in the cell factory *Saccharomyces cerevisiae* and the food spoilage yeast *Zygosaccharomyces bailii,* remarkably tolerant to weak acid food preservatives. [2]

Methods: Chemogenomic, transcriptomic, (quantitative- and phospho-) proteomic,lipidomic analyses and genome sequencing and analysis coupled with bioinformatics tools and molecular and cellular biology studies are explored to unveil genome-wide adaptive response programs and tolerance/susceptibility determinants to single and multiple relevant stresses.

Conclusions: The involvement of genes, proteins and signaling pathways in yeast adaptation and tolerance to relevant stresses is being elucidated based on genome-wide strategies. Those are candidate molecular targets for genetic manipulations to endure yeast cells against multiple stresses expected to occur during biotechnological and food industry processes.

References: [1] dos Santos SC, Sá-Correia, I, Curr Opin Biotech, 2015, 33: 183–191; [2] Palma M *et al*, BMC Genomics. 2017, 18: 75.

FEMS7-0349
FEMS-ALAM-SEM workshop: Crosstalk between biofilm formation and applied microbiology

ROLE OF BIOFILM FORMATION ON ANTIBIOTIC RESISTANCE IN PSEUDOMONAS AERUGINOSA

J. Ayala[1], C. Aguilera[2], A. Ropy[3]
[1]Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid, Spain
[2]Universidad de la Frontera,
Facultad de Medicina- Departamento de Ciencias Preclínicas- Unidad de Microbiología, Temuco, Chile
[3]Fayoum University, Faculty of Science- Department of Chemistry, Fayoum, Egypt

Community and nosocomial infections by *Pseudomonas aeruginosa* still create a major therapeutic challenge. The resistance of this opportunist pathogen to β-lactam antibiotics is determined mainly by production of the inactivating enzyme AmpC, a class C cephalosporinase with a regulation system more complex than those found in members of the Enterobacteriaceae family. This regulatory system also participates directly in peptidoglycan turnover and recycling. One of the regulatory mechanisms for AmpC expression, recently identified in clinical isolates, is the inactivation of LMM-PBP4 (Low-Molecular-Mass Penicillin-Binding Protein 4), a protein whose catalytic activity on natural substrates has remained uncharacterized until now. We carried out in vivo activity trials for LMM-PBP4 of Pseudomonas aeruginosa on macromolecular peptidoglycan of *Escherichia coli* and *Pseudomonas aeruginosa*. The results showed a decrease in the relative quantity of dimeric, trimeric and anhydrous units, and a smaller reduction in monomer disaccharide pentapeptide (M5) levels, validating the occurrence of D,D-carboxypeptidase and D,D-endopeptidase activities. Under conditions of induction for this protein and cefoxitin treatment, the reduction in M5 is not fully efficient, implying that LMM-PBP4 of *Pseudomonas aeruginosa* presents better behavior as a D,D-endopeptidase. Kinetic evaluation of the direct D,D-peptidase activity of this protein on natural muropeptides M5 and D45 confirmed this bi-functionality and the greater affinity of LMM-PBP4 for its dimeric substrate. A three-dimensional model for the monomeric unit of LMM-PBP4 provided structural information which supports its catalytic performance. So, LMM-PBP4 of *Pseudomonas aeruginosa* is a bi-functional enzyme presenting both D,D-carboxypeptidase and D,D-endopeptidase activities; the D,D-endopeptidase function is predominant. Our study provides unprecedented functional and structural information which supports the proposal of this protein as a potential hydrolase-autolysin associated with peptidoglycan maturation and recycling. The fact that mutant PBP4 induces AmpC, may indicate that a putative muropeptide-subunit product of the DD-endopeptidase activity of LMM-PBP4 could be a negative regulator of the pathway. Role of this muropeptide on *ampR*-dependent biofilm induction will be discussed. Incubation of *Pseudomonas aeruginosa* with 5x MIC of imipenem caused the development of spherical shaped cells, which are able to recover after drug elimination We traced the effect of imipenem on activities of Penicillin Binding Proteins (PBPs), DD-endopeptidases, DD-carboxypeptidases, LD-carboxypeptidases, and peptidoglycan composition in spheroplast and rod-shaped *Pseudomonas aeruginosa*. We found that the rod-shaped mutant PAOΔ*dacB*Δ*dacC*Δ*pbpG*Δ*ampC* displayed the same behavior as the wild type PAO1 in spheroplast generation and rod shape recovery beyond imipenem treatment and elimination, respectively. Also, spheroplasts from both strains had similar changes in their PG composition like increase in levels of M3 and anhydromuropeptides. Additionally, spheroplast PG had a decrease in levels of M5, D44, D45 and D-D muropeptides. These changes in PG composition can be explained by imipenem inhibition of DD-endopeptidase and DD-carboxypeptidase activities. This proposal was confirmed by quantification of PBPs by Bocillin-FL test and qRT-PCR which revealed disappearance of many PBPs bands from samples of spheroplast membranes. Our data suggests that the large increase in M3 muropeptides was due to the uncompensated activity of LD-carboxypeptidases. Also, these results demonstrate that activities of LMM-PBP4, LMM-PBP5 and LMM-PBP7 are not essential for recovery of rod shape in imipenem-induced spheroplasts in *Pseudomonas aeruginosa*. These data contributes to

understanding of the regulatory aspects of resistance to β-lactam antibiotics in this bacterial model, and provide a link with other biological processes.

FEMS7-0498
FEMS-ALAM-SEM workshop: Crosstalk between biofilm formation and applied microbiology

SYNERGISTIC EFFECTS AND INHIBITION OF BIOFILM FORMATION OF OCELLATIN PEPTIDES IN PSEUDOMONAS AERUGINOSA

L.J. Bessa[1], J.R.S.A. Leite[1,2], P. Eaton[1], P. Gameiro[1]
[1]Requimte, LAQV, Departamento de Química e Bioquímica,
Faculdade de Ciências da Universidade do Porto, Porto, Portugal
[2]Área de Morfologia, Faculdade de Medicina, Universidade de Brasília, UnB, Brasília, Brasil

Backgrounds

Antimicrobial resistance is putting at stake the effective treatment of many infections. It is therefore pertinent to search for new antimicrobials, inhibitors of biofilms and agents able to restore or potentiate the activity of existing antibiotics. Five peptides (Ocellatins-PT2 to –PT6) isolated from the skin secretion of the frog *Leptodactylus pustulatus* and previously characterized [1], were used in this study.

[1] Marani MM *et al.* J. Nat. Prod. 2015;78(7):1495-504. doi: 10.1021/np500907t.

Objectives

Assess potential synergies between peptides and antibiotics against multidrug-resistant isolates. Study the ability of peptides in inhibiting the biofilm proliferation of *Pseudomonas aeruginosa* isolates.

Methods

The combination of peptides and clinically important antimicrobial drugs was assayed through the disc diffusion method on agar, against multidrug-resistant isolates of *Escherichia coli*, *Staphylococcus aureus* and *P. aeruginosa*. Potential synergy between Ocellatins and ciprofloxacin or ceftazidime against *P. aeruginosa* were further confirmed using a broth microdilution checkerboard method. The inhibition of *P. aeruginosa* biofilm formation by Ocellatin-PT3 was evaluated through 1) the crystal violet assay and 2) microscopic visualization by atomic force microscopy (AFM). The minimum biofilm inhibitory concentration (MBIC) was also determined.

Conclusions

Ocellatin-PT3 showed synergy with ciprofloxacin and ceftazidime against multidrug-resistant isolates of *P. aeruginosa*. The mechanisms behind these synergies must be further explored, however, hypothetically, we can suppose these peptides can increase the membrane permeability, allowing an easier entrance of the antibiotics into the cell. Equally, Ocellatin-PT3 was shown to be very promising in preventing the proliferation of a 24-h mature *P. aeruginosa* biofilm, the MBIC being 8× the MIC.

FEMS7-3084
FEMS-ALAM-SEM workshop: Crosstalk between biofilm formation and applied microbiology

ANTIBIOTIC SUSCEPTIBILITY AND BIOFILM FORMATION OF URINARY ISOLATES OF ENTEROCOCCUS FAECALIS FROM PATIENTS IN CONSULTATION AT THE CHARLES NICOLLE HOSPITAL, TUNIS

R. Chettaoui[1], *G. Mayot*[1], *I. Boutiba*[2], *P. Di Martino*[1]
[1]*Université de Cergy-Pontoise, Laboratoire ERRMECe, Cergy-Pontoise, France*
[2]*Université de Tunis, Laboratoire de Bactériologie de l'hôpital Charles Nicolle de Tunis, Tunis, Tunisia*

Backgrounds

Enterococcus faecalis is a major nosocomial uropathogen.

Objectives

We report here the epidemiological investigation of drug resistance and *in vitro* biofilm formation among *E. faecalis* strains isolated from patients in consultation for presumptive urinary tract infection in the Charles Nicolle Tunisian hospital from October 2015 to March 2016.

Methods

The microbial isolates obtained after urine cultivation on CLED agar were identified by Gram staining, oxidase or catalase reaction, and conventional biochemical tests. The susceptibility to antibiotics was determined by the disk diffusion method according to recommended French standard. Biofilm formation was quantified using the microtiter plate assay and crystal violet staining.

Conclusions

Of the 120 samples analysed, 39% were positive for *Esherichia coli*, 23% for *Enterococcus faecalis*, 10% for *Proteus mirabilis*, 9% for *Candida albicans*, 8% for *Staphylococcus aureus*, 7% for *Enterococcus faecium*, and 5% for *Klebsiella pneumoniae*. The 28 corresponding *E. faecalis* isolates showed systematic resistance to oxacillin, cefalotin, and clindamycin; moderate rates of resistance to pristinamycin, ciprofloxacin, streptomycin, tetracycline and erythromycin; and low resistance frequencies to chloramphenicol, ampicillin and linezolide. All isolates showed susceptibility or intermediate susceptibility to imipenem, rifampicin, teicoplanin and vancomycin. Multi-resistance to at least three different classes of antibiotics was detected in 14 isolates. The patients infected by *E. faecalis* were predominantly men with a mean age of 46.8 years, with an underlying disease. All the 28 *E. faecalis* isolates effectively formed biofilms to varying degrees. The correlation between biofilm formation, multi-resistance to antibiotics, and underlying diseases is discussed.

FEMS7-1183
FEMS-ALAM-SEM workshop: Crosstalk between biofilm formation and applied microbiology

PEL EXOPOLYSACCHARIDE MACHINERY IS INVOLVED IN BIOFILM ARCHITECTURE DEVELOPMENT BY ACIDITHIOBACILLUS THIOOXIDANS

M. Diaz[1], N. Guiliani[1]
[1]University of Chile, Faculty of Sciences - Department of Biology, Santiago, Chile

Backgrounds

Acidophilic bacterial communities from natural environments frequently occur as biofilms, slimes and other macroscopic growth formations on mineral surfaces, streams and water bodies. Several reports emphasized the taxa of *Acidithiobacillus* as a predominant structural and active player in acidophile communities. In addition, the molecular understanding of biofilm formation by *Acidithiobacillus* spp. has been pointed out to design biological strategies to improve the efficiency of bioleaching process. We have previously described the second messenger cyclic diguanylate (c-di-GMP) as a key player for biofilm formation by several *Acidithiobacillus* species.

Objectives

In the course for the characterization of c-di-GMP effectors, we have recently identified a complete *pel*-like operon in *Acidithiobacillus* species only capable to oxidize reduced inorganic sulfur compounds such as *At. caldus* and *At. thiooxidans*. Thus the main goal of this work was to characterize the structural role of Pel exopolysaccharide in biofilm formation by *At. thiooxidans*.

Methods

Here results obtained from biochemical, genetic, microscopy and proteomic analysis will be reported. By using total RNA obtained from planktonic and adhered sulfur-grown cells, transcriptomic analysis revealed that genes belonging to *pel*-like operon are overexpressed in adhered cells. Mutagenesis experiments indicated that c-di-GMP effector PelD is involved in the regulation of biofilm architecture in this microorganism. Proteomic experiments are actually running to characterize long filamentous structures overexpressed in *pelD*- null-mutant strain.

Conclusions

Our current results point out a pivotal role for Pel exopolysaccharide machinery in the attachment to solid energy substrates by *Acidithiobacillus* sulfur-oxidizing species.

This work was supported by Fondecyt 1120295 and 1160702.

FEMS7-3148
FEMS-ALAM-SEM workshop: Crosstalk between biofilm formation and applied microbiology

DEVELOPMENT OF NOVEL ANTIMICROBIAL AND ANTIBIOFILM PEPTIDES FOR FOOD APPLICATIONS

V. Ghate[1], T. Stern[1], Z. Hayouka[1]
[1]The Hebrew University of Jerusalem - The Faculty of Agriculture, Institute of Biochemistry- Food Science and Nutrition, Rehovot, Israel

Backgrounds

Food-borne diseases have a major health, social, economic and industrial impact. As food production becomes increasingly automated, the number of surfaces which food come into contact with and the potential for cross-contamination increases. Food contamination leads to spoilage and growth of pathogenic microorganisms. Biofim is defined as structured aggregation of surface-attached bacteria encased in an extracellular matrix that leads to bacterial resistance to antibiotics. The difficulty of successfully treating biofilm and the increasing resistance of microbes to traditional treatments demands for the discovery of compounds with novel mode of action to tackle this threat. Host-Defense Peptides (HDPs) are produced by eukaryotes as part of the innate immune response to bacterial infection. These agents represent a potential source of inspiration for development of new antibacterial agents but less is known about their ability to prevent biofilm formation.

Objectives

Designing new approaches to inhibit microbial food contamination and bacterial biofilm formation while maintaining quality, freshness, and safety are required.

Methods

The broad molecular diversity among HDPs suggests that their activity is not tightly coupled to specific features of amino acid sequence or peptide conformation. This situation has inspired us to develop sequence-random hydrophobic-cationic peptides that display antibacterial behavior. We employed solid-phase synthesis in an unconventional way to generate peptide mixtures that contain one type of hydrophobic residue and one type of cationic residue. So far we have demonstrated their broad antimicrobial activity and selectivity towards several pathogenic bacteria. In the current talk we will present our findings on the ability of our compounds to prevent biofilm formation either by inhibiting the initial bacterial adhesion to the surface or by the removal of the established biofilm.

Conclusions

According to our findings the random peptide mixtures are able to control and manage biofilm and might be use as a lead biofilm inhibitor to prevent food contamination.

FEMS7-1813
FEMS-ALAM-SEM workshop: Crosstalk between biofilm formation and applied microbiology

ELICITING ELECTRON TRANSFER IN ACIDOGENIC BIOCATHODES FROM CHANGES IN THE BIOFILM MICROBIOME

E. Perona-Vico[1,2], R. Blasco-Gómez[2], S. Puig[2], J. Colprim[2], L. Bañeras[1]
[1]Universitat de Girona, Institut d'Ecologia Aquàtica, Girona, Spain
[2]LEQUIA, Institute of the Environment. Universitat de Girona, Girona, Spain

Backgrounds

Microbial electrosynthesis (MES) is acquiring importance as an environment-friendly platform for the production of commodity chemicals from CO_2. In MES, direct and a mediated electron transfer events have been proposed as initial electron harvesting mechanisms. In this sense, a better understanding of the cathode microbiome will help to elucidate which microbial species are likely participating in electron capture.

Objectives

This study aimed to determine the composition of the bacterial community present in electrotrophic biofilms with increasing operation times. Our idea was to test the bacterial populations that remained firmly attached on the electrode, being at the forefront of electron harvesting in electrode-driven acidogenesis.

Methods

Biocathodes basically consisted of a carbon cloth electrode poised at -0.8 V (vs. standard hydrogen electrode, SHE), operated in a two-chambered bioelectrochemical system. Microbial diversity of the biofilms and the bulk liquid was analysed using barcoded amplicon massive sequencing (Illumina®), after two and six months of operation.

Conclusions

Microbial diversity was low in newly developed biofilms (H'=2.8) and significantly increased in older ones (H'=3.5). Despite this change, putative electrotrophic bacteria, such as *Clostridium* and *Sporomusa*, occurred at a high relative abundance (33.83% and 18.18% of sequences, respectively), and appeared from the very beginning of MES operation. The relative abundance of these species **i**ndicates a major involvement of direct electron transfer mechanisms. Electrotrophic bacteria were partially substituted by non-electroactive bacteria (*Cellulomonas* and *Rhodocyclaceae*) probably growing at the expenses of electrically generated acetate. The presented results indicate that preventing growth of heterotrophic bacteria is mandatory to up-scaling MES towards application.

FEMS7-0308
Food microbiology-production

BIOCHEMICAL AND FUNCTIONAL ANALYSIS OF MODIFIED HEPTOSES FROM THE CAPSULE OF CAMPYLOBACTER JEJUNI

C. Creuzenet[1], A. Wong[1], D. Lange[1], S. Houle[2], H. Barnawi[1], G. Shaw[3], C. Dozois[2]
[1]Western University, Microbiology and Immunology, LONDON, Canada
[2]Institut National de la Recherche Scientifique Institut Armand-Frappier, na, Laval, Canada
[3]Western University, Biochemistry, LONDON, Canada

Backgrounds

Campylobacter jejuni is a commensal in poultry but also a human bacterial pathogen that is a predominant cause of enteritis worldwide. Its capsule is an external polysaccharide layer important for colonization and virulence. In most strains, the capsule comprises a modified heptose.

Objectives

To investigate the biological roles and biosynthetic pathways of capsular heptoses.

Methods

This work uses knockout mutagenesis, phenotypic analyses and many types of biochemical assays for carbohydrate analyses and enzymology.

Conclusions

We deciphered the biosynthesis pathways for 6-deoxy-D-*altro*-heptose of strain 81-176 and 3,6-OMe-L-*gluco*-heptose of strain NCTC 11168. This allowed a direct comparison of novel C3/C5 epimerases and C4 reductases involved in these pathways. We determined the activity of 7 enzymes, revealing unexpected functions and specificities and complex regulatory loops. Knockout mutagenesis studies of heptose modifying genes in strain NCTC 11168 showed that heptose modification is not necessary for capsule synthesis but affects bacterial resistance to serum and bile salts, biofilm formation, adhesion to intestinal epithelial cells and their invasion. The mutants also showed slightly decreased phagocytosis by macrophages but no defect for survival inside macrophages. We also demonstrate that heptose modifying genes are important for colonization and persistence of *C. jejuni* in chicken. These findings suggest that fine tuning the capsule composition via heptose modification contributes to host pathogen interactions.

This work provides grounds for the elucidation of similar pathways of other pathogens. It provides new molecular tools to synthesize carbohydrate antigens useful for vaccination and to screen for enzymatic inhibitors with antibacterial effects that could be used to decrease Campylobacteriosis.

FEMS7-0871
Food microbiology-production

RESISTANCE TO THE BACTERIOCIN LCN972 AS DECIPHERED BY GENOME SEQUENCING

S. Escobedo[1], Ö.C.O. Umu[2], A. Rodríguez[1], D.B. Diep[2], B. Martinez[1]
[1]IPLA-CSIC, Technology and Biotechnology of Dairy Products, Villaviciosa-Asturias, Spain
[2]Norwegian University of Life Sciences, Faculty of Chemistry- Biotechnology and Food Science, Ås, Norway

Backgrounds

In view of the current threat of antibiotic resistance, new antimicrobials with low risk of resistance development are demanded. Lcn972 is a lactococcal bacteriocin that inhibits septum formation in dividing target cells by binding to the cell wall precursor lipid II. Moreover, it has a species-specific spectrum of activity, making Lcn972 an attractive template to develop or improve existing antibiotics.

Objectives

Our aim was to identify mutations underlying resistance to Lcn972.

Methods

Whole-genome sequencing (WGS) of the sensitive *Lactococcus lactis* MG1614 (MIC=10 AU/ml) and two Lcn972-resistant (Lcn972R) derivatives D1 and D1-20 with MICs of > 320 AU/ml and 80 AU/ml, respectively, was performed using the Illumina MiSeq platform and Rapid Annotation Subsystem Technology (RAST) server. Sequence reads were assembled with SPAdes 3.1.0. CONTIGuator and Mauve software tools were used for contigs mapping and comparison over the reference genome.

Conclusions

Two previously identified mutations in *L. lactis* D1 and D1-20 were mapped and confirmed by WGS: 1) a 22.6-kbp deletion enclosing genes involved in maltose metabolism, the two component system (TCS) F and the phage infection protein (Pip), and 2) insertion of IS*981* that activates the promoter of *llmg_2447* coding for a putative anti-ECF sigma factor. Additionally, a new mutation was identified, entailing insertion of IS*905* in the promoter of the operon *llmg_0186_celB* that specifies the IIC component of the cellobiose phosphotransferase PTS system. The insertion took place between the putative -35 and -10 promoter regions and the consequences, in terms of promoter activity, are currently under investigation.

FEMS7-2334
Food microbiology-production

DOES THE FOOD PRODUCTION HAS A KEY ROLE IN THE ANTIBIOTIC RESISTANCE BURDEN?

L. Peixe[1], *P. Antunes*[2], *C. Novais*[3]
[1]*Faculdade de Farmácia da Univ. do Porto, Faculdade de Farmácia da Univ. do Porto, Porto, Portugal*
[2]*Faculdade das Ciências da Nutrição da Univ. do Porto, Faculdade das Ciências da Nutrição da Univ. do Porto, Porto, Portugal*
[3]*Faculdade de Farmacia da Univ. do Porto, Faculdade de Farmacia da Univ. do Porto, Porto, Portugal*

Increased antibiotic resistance in nearly all bacteria causing infections challenges the eradication of human infections raising morbidity and mortality. Human exposition to antibiotic-resistant bacteria could occur through a number of routes such as human-to-human spread, environment, direct contact with animals, and also through the food chain. Foods from many different animal sources and in all stages of processing contain abundant quantities of resistant bacteria and resistance genes.

Supported on different studies it has been possible to elucidate the link to animal production of different antibiotic-resistant species, namely of *E. coli* and *Salmonella* resistant to extended-spectrum β-lactams. More recently, the global spread of microorganism with MCR-1 (a protein conferring resistance to colistin, a last resort antibiotic) and the presence of such organisms in livestock raise concerns about the possibility of their dissemination throughout food. Consequently, there is a need for interventions to reduce the use of antibiotics in the animal production and of quick methods for tracking antibiotic-resistant clones in order to assist in the formulation of measures to prevent their potential spread.

FEMS7-0827
Food microbiology-production

ANTIMICROBIAL PEPTIDES AND THE EVOLUTION OF RESISTANCE

D. Ramon[1]
[1], *Paterna, Spain*

During the last five years an increasing amount of information about the composition of the human gut microbiome has been generated. Most of this information has been obtained from healthy volunteers but also description of gut microbiomes from people suffering different pathologies, mainly related with gastrointestinal disorders, has been established.

In our company we have studied the gut microbiome of patients suffering several disorders (celiac disease, atopic dermatitis and psoriasis, unfertility). Changes in the profiles of microbial communities have been detected in all the cases. Taking into account this information we have generated different cocktails of probiotic strains that have been used in clinical trials. The results of these clinical trials are very promising opening the way for the creation of nutritional supplements or functional foods containing these probiotics.

This strategy based on microbiome analysis is actually used for the study of other pathologies in our company. All these results will be presented in the congress.

FEMS7-2072
Food microbiology-production

BIFIDOBACTERIUM LONGUM IPLA20022 IS ABLE TO COUNTERACT THE CYTOTOXIC EFFECT OF CLOSTRIDIUM DIFFICILE UPON HT29 COLONOCYTES

L. Valdés-Varela[1], M. Gueimonde[1], B. Sánchez[1], P. Ruas-Madiedo[1]
[1]IPLA-CSIC, Microbiology and Biochemistry of Dairy Products, Villaviciosa-Asturias, Spain

Backgrounds

Infections caused by *Clostridium difficile* are increasing and constitute a problem due to the high morbidity and mortality rates in groups of risk, being the main aetiological agent of antibiotic-associated diarrhoea. This opportunistic pathogen is a common inhabitant of the intestinal microbiota but starts to proliferate after a microbiota dysbiosis caused by antibiotic treatment. The major virulence factor is the production of different toxins, mainly A and B. In previous works we have selected the strain *Bifidobacterium longum* IPLA20022 as a probiotic candidate being able to *in vitro* reduce the toxicity of *C. difficile* LMG21717 producing both types of toxins.

Objectives

Our aim is to gain insight into the potential mechanisms involved in the ability of IPLA20022 strain to reduce the cytotoxic effect of *C. difficile* upon an intestinal epithelial monolayer obtained from HT29 cells.

Methods

Different cellular fractions were obtained from *B. longum* IPLA20022 and were incubated with *C. difficile*. Afterwards, the cell-free supernatants were tested for their anti-clostridial capability upon HT29 cells using the RTCA (real time cell analyser) technology following the integrity of the intestinal monolayer; the remaining toxins were analyzed by means of ELISA tests. Those bifidobacterial fractions showing capability to reduce the toxicity of *C. difficile* were analyzed by means of proteomic techniques.

Conclusions

Preliminary results show that extracellular factors from *B. longum* IPLA20022 are involved in the reduction of clostridial cytotoxicity. This opens new opportunities for the therapeutically application of the strain, or their metabolites, for attenuating symptoms of *C. difficile* infection.

FEMS7-2376
Food microbiology-production

SURVEY OF THE METABOLICALLY ACTIVE BACTERIAL MICROBIOME OF LYMPH NODES IN SLAUGHTER PIGS, CONFIRMED BY CULTIVATION OF VIABLE BACTERIA AND AMPLICON PYROSEQUENCING

S.U. Wetzels[1,2], *M. Dzieciol*[1], *M. Wagner*[1,2], *S. Schmitz-Esser*[2,3], *E. Mann*[1,2]
[1]*Institute for Milk Hygiene- Milk Technology and Food Science,*
Department for Farm Animals and Veterinary Public Health- University of Veterinary Medicine Vienna, Vienna, Austria
[2]*Research Cluster 'Animal Gut Health', University of Veterinary Medicine Vienna, Vienna, Austria*
[3]*Department of Animal Science, Iowa State University, Ames, USA*

Backgrounds

The exploration of microbiota in lymphatic organs is relevant for tracing of microbial translocations and cross-contamination during slaughter.

Objectives

This study aimed (1) to characterize the bacterial diversity of unreactive ileocecal lymph nodes (ICLNs) of slaughter pigs and to investigate microbiome shifts in enlarged, purulent or granulomatous ICLNs, (2) to characterize viable, cultivable bacteria, and (3) to define the metabolically active bacterial microbiota in lymph nodes.

Methods

Three approaches were used: (1) 16S rRNA gene pyrosequencing from 32 lymph nodes, (2) collection of 209 isolates with aerobic and anaerobic cultivation and near full-length 16S rRNA gene sequencing, and (3) cDNA pyrosequencing, to investigate the lymph node's bacterial metabolically active fraction, including non-cultivable bacteria.

Conclusions

(1) Pyrosequencing yielded 175,313 sequences, clustering into 650 operational taxonomic units (OTUs). OTUs were assigned to 239 genera and 11 phyla. Beside of a highly diverse bacterial community we observed significant shifts in pathologically altered ICLNs. (2) *Proteobacteria* and *Firmicutes* were the most abundant phyla. Purulent and granulomatous ICLNs tended to contain more *Proteobacteria* than asymptomatic and enlarged ICLNs. Isolates could be assigned to 25 species belonging to 17 genera. (3) The number of detected OTUs per lymph node varied highly (23-171 OTUs). *Serratia proteamaculans* (best type strain hits) were most abundant (41.8%). We conclude that (i) lymphatic organs harbor a high diversity of metabolically active bacteria, (ii) the occurrence of viable bacteria in lymph nodes is not restricted to pathological processes and (iii) lymphatic tissues may serve as contamination source in pig slaughterhouses.

FEMS7-1356
Vaccines

NEW TUBERCULOSIS VACCINES: FROM THE DISCOVERY TO CLINICAL EVALUATION IN HIGH-BURDEN COUNTRIES

M. Carlos[1]
[1]*Universidad de Zaragoza, Microbiologia, Zaragoza, Spain*

The only vaccine in use against TB in humans today is BCG, a live attenuated vaccine derived from the bovine pathogen *Mycobacterium bovis*. BCG presents variable protection against pulmonary forms of TB. Genomic comparative studies have shown loss of a number of major mycobacterial antigens and about 23% of *M. tuberculosis*-specific human T-cell epitopes in BCG relative to the human pathogen *Mycobacterium tuberculosis*. MTBVAC is a new live tuberculosis vaccine based on a genetically attenuated *phoP-fadD26*-deletion mutant of *M. tuberculosis*. The presence of stable deletion mutations in two independent virulence genes abrogates the risk of reversion to virulence. MTBVAC has shown a comparable or superior safety and immunogenicity profile to BCG in different preclinical animal models including new-born mice model (Arbues *et al* Vaccine 2013 and Broset *el al* mBio 2015, Aguilo et al JID 2016).

A first-in-human MTBVAC clinical trial was recently completed successfully in healthy adults in Lausanne, Switzerland sponsored by Biofabri (NCT02013245) (Spertini *et al* LRM 2015). In this trial, when MTBVAC was given at the same dose as BCG ($5x10^5$ CFU), there were more responders in the MTBVAC group than in the BCG group, with a greater frequency of polyfunctional $CD4^+$ central memory T cells. MTBVAC is the first live-attenuated *M. tuberculosis* vaccine to enter clinical trials and to date has shown a comparable safety profile to BCG. A notable finding in the first trial was the absence of ESAT-6 and CFP-10-specific T cell responses at the end of the study, suggesting that interferon-g release assays (IGRAs) could be utilized as study endpoints in future efficacy trials to test efficacy against *M. tuberculosis* infection. The immunogenicity data show that MTBVAC is at least as immunogenic as BCG. Altogether these data supported the advanced clinical development in high-burden countries where TB is endemic. A Dose-escalation safety and Immunogenicity study to compare MTBVAC to BCG in newborns with a safety arm in adults is currently ongoing in South Africa sponsored by Biofabri (NCT02729571). MTBVAC is developed with the goal to provide improved efficacy over BCG for use in new-borns, adolescents and adults as a preventive strategy against tuberculosis in high-burden countries (Arregui *et al* PeerJ 2016).

Funding: Biofabri, TuBerculosis Vaccine Initiative (TBVI). BIO2014 5258P and TBVAC2020 643381 Spanish and European grants.

References:

-. Aguilo N, Uranga S, Marinova D *et al* MTBVAC vaccine is safe, immunogenic and confers protective efficacy against *Mycobacterium tuberculosis* in newborn mice. Tuberculosis **2016**; 96:71-4.

-. Arbues A, Aguilo JI, Gonzalo-Asensio J, *et al.* Construction, characterization and preclinical evaluation of MTBVAC, the first live-attenuated *M. tuberculosis*-based vaccine to enter clinical trials. Vaccine **2013**;

-. Arregui S, Sanz J, Marinova D, *et al* . On the impact of masking and blocking hypotheses for measuring the efficacy of new tuberculosis vaccines. PeerJ. 2016 Feb 11;4:e1513.

-. Broset E, Martín C, Gonzalo-Asensio J.Evolutionary landscape of the Mycobacterium tuberculosis complex from the viewpoint of PhoPR: implications for virulence regulation and application to vaccine development. MBio. 2015 Oct 20;6(5):e01289-15.

-. Spertini F, Audran R, Chakour R, *et al.* Safety of human immunisation with a live-attenuated *Mycobacterium tuberculosis* vaccine: a randomised, double-blind, controlled phase I trial. The lancet Respiratory medicine **2015**; 3:953-62.

FEMS7-1289
Vaccines

MULTIVALENT COMBINATION OF MTBVAC-BASED VACCINES CONSTRUCTED IN THREE WIDESPREAD LINEAGES OF MYCOBACTERIUM TUBERCULOSIS

I. Perez[1], *C. Martin*[1], *J. Gonzalo-Asensio*[1]
[1]*Universidad de Zaragoza, Microbiología- Medicina Preventiva y Salud Pública, Zaragoza, Spain*

Backgrounds

Despite the existence of a vaccine against TB (BCG), this disease still causes 1.8 millions of deaths per year *(WHO, 2016)*. BCG is based on the cow pathogen *Mycobacterium bovis* and its protection against pulmonary TB in adults is variable. For this reason it has been constructed a new live attenuated candidate vaccine, MTBVAC, based on the human pathogen *M. tuberculosis* from lineage 4 (Euro-American). MTBVAC is currently in clinical trials (phase Ib, NCT02729571) and it is based on the deletions of two virulence related genes (*phoP* and *fadD26*) *(Arbués, Vaccine, 2013)*.

Objectives

Construction the same deletions of MTBVAC in lineage 2 (Asian Beijing) and lineage 3 (African-Indian) of *M. tuberculosis*. These are modern lineages, with together with lineage 4, are highly distributed and the principal responsible of the transmission of TB in humans. Lineage-dependent protection in mouse model will be evaluated.

Methods

Suicide plasmids which contains *phoP* or *fadD26* disrupted by hygromycin resistence gene and a counter-selectable marker will be used. The antibiotic marker will be eliminated using a γδ-resolvase which acts on res sites flanking the resistance marker.

Conclusions

Constructions of MTBVAC-like vaccines are in progress. Phenotypes previously demonstrated in MTBVAC will be studied. These include: lack of cell-wall lipids phthiocerol dimycocerosates (PDIM), diacyltrehaloses and polyacyltrehaloses (DAT/PAT), down-regulated expression of *pks2*, *pks3*, *espA* among others *(Gonzalo-Asensio, PLoS One, 2008*; *Solans PLoS Pathog, 2014)* and the inability to secrete ESAT-6 *(Gonzalo-Asensio, Proc Natl Acad Sci U S A, 2014).*

FEMS7-2288
Vaccines

SEARCHING FOR NEW TAGGED BRUCELOSIS VACCINES AND ASSOCIATED DIAGNOSTIC TESTS

E. Martínez-Gómez[1], Y. Gil-Ramírez[1], J.M. Blasco[2], P. Muñoz[2], M.J. de Miguel[2], A. Zúñiga-Ripa[1], I. Moriyón[1], M. Iriarte[1], R. Conde-Álvarez[1]
[1]Universidad de Navarra, Instituto de Salud Tropical. Microbiology and Parasitology, Pamplona, Spain
[2]Centro de Investigación Tecnología Agroalimentaria CITA del Gobierno de Aragón, Unidad de Sanidad Animal, Zaragoza, Spain

Backgrounds

Brucellosis, a zoonosis caused essentially by *B. melitensis* and *B. abortus*, infects livestock and humans causing heavy economic losses. A complete lipopolysaccharide (LPS) is critical for their virulence. Its O-chain is a homopolymer of N-formyl-perosamine, and carries the immunodominant epitopes.

The only useful vaccines to control brucellosis are smooth (S) live attenuated: *B. melitensis* Rev1 (small ruminants), and *B. abortus* S19 (bovine). However, the differentiation between infected and vaccinated animals (DIVA problem) is difficult, when performed soon after vaccination, since the serological diagnostic tests detect the antibody response against the O-chain that is present in both vaccines and wild-type strains.

Objectives

To develop a *Brucella* tagged vaccine and associated DIVA diagnostic tests by modifiying *Brucella* O-chain.

Methods

We tagged wild-type *B. abortus* LPS by inserting into the chromosome *wbdR,* a gene that encodes an acetyltransferase that adds an acetyl group to the perosamine of *E.coli* O157 O-chain (Ba::Tn7wbdR). We also combined insertion of *wbdR* with deletion of gene *wbkC* encoding the *Brucella* perosamine formyltransferase (Ba::Tn7wbdRΔwbkC). Both Ba::Tn7wbdR and Ba::Tn7wbdRΔwbkC carry LPS with O-chains that contain new N-acetyl perosamine-associated epitopes that are not present in *B. abortus* wild-type LPS. The strains were tested in mice. We also tagged *B. melitensis* Rev 1 vaccine strain with *wbdR* (Rev1::*wbdR)* and tested in sheep. Finally, we developed two associated serological tests (agglutination test and iELISA) for DIVA purposes.

Conclusions

The DIVA tests developed (an agglutination test and an iELISA with S-LPS antigen obtained from Ba::Tn7wbdR) allow the differentiation of mice infected with Ba-parental strain from those infected with Ba::Tn7wbdRΔwbkC or Ba::Tn7wbdR. Moreover these results were confirmed in a preliminary study in the natural host (sheep) using the Rev1::*wbdR*. Thus, introducing *wbdR* into *Brucella* vaccinal background might represent a suitable strategy to solve the DIVA problem in brucellosis.

FEMS7-3288
Bergey's Manual Trust: "Higher taxonomy of uncultivated microorganisms"

GENOME-SCALE DATA RESOLVE PHYLOGENY AND CLASSIFICATION OF VARIOUS BACTERIAL PHYLA

M. Göker[1]
[1]*Leibniz Institute DSMZ, Phylogenomics, Braunschweig, Germany*

Due to the staggering advances in sequencing technology it should now be possible, in principle, to base phylogeny and classification of microorganisms on information from genome-scale data. However, genome sequencing alone will not yield an adequate taxonomic classification of *Archaea* and *Bacteria*. Adequate methods are needed to infer phylogenies from genome-scale data and taxonomic classifications from these phylogenies. This talk first recapitulates the goal of phylogenetic classification and its historic origin. I will then briefly discuss contemporary approaches in microbial systematics in conflict with this goal and comment on the relationship between phenotypic and genomic data with respect to phylogenetic classification. Next, it is discussed why phylogenies and classifications should be derived from genome-scale data despite the prevalence of horizontal gene transfer, which had led some authors to instead conclude that both the idea of a microbial tree of life and the hierarchical classification of prokaryotes should be dismissed. The lack of genome sequences for type strains of major lineages of prokaryotes is, of course, a real obstacle for a genome-based classification of microorganisms, but phylogeny-driven microbial genome sequencing projects such as the Genomic Encyclopaedia of Archaea and Bacteria (GEBA) and the One Thousand Microbial Genomes (KMG) project, which are briefly introduced, are currently solving this issue. Finally, examples for genomic approaches to obtain a phylogenetic classification of distinct phyla of prokaryotes are provided, making use of the data from such projects.

FEMS7-3307
Bergey's Manual Trust: "Higher taxonomy of uncultivated microorganisms"

CALIBRATING UNCULTURED MICROBIAL DIVERSITY WITH A GENOME-BASED TAXONOMY

P. Hugenholtz[1]
[1]*University of Queensland, Australia*

Despite decades of concerted microbiological endeavour, the great majority of microorganisms have not been cultured and characterised. This so-called "microbial dark matter" is now being revealed at an ever increasing rate by sequence-based culture independent methods. In the past few years, thousands of near complete genomes of uncultured microbes have been assembled from sequence data obtained directly from environmental and clinical sources providing the opportunity to fully articulate microbial diversity for the first time. Current estimates suggest that cultured microorganisms only capture ~15% of total microbial diversity based on evolutionary divergence of marker genes. Here we use a genome-based taxonomy founded on the existing classification of cultured organisms to calibrate and quantify the taxonomic diversity of microbial dark matter.

FEMS7-3276
Bergey's Manual Trust: "Higher taxonomy of uncultivated microorganisms"

TO BE OR NOT TO BE A MODERN AND UNIVERSAL TAXONOMY, THAT IS THE QUESTION

R. Rossello-Mora[1]
[1], *Spain*

If we want to describe the vast majority of the living diversity we cannot neglect the relevance of the methodological approach improvements. Thus Taxonomy for Prokaryotes needs to modernize by adapting the new high throughput methodologies to construct a classification that responds to the needs of a wide scientific community. Mass spectrometry for phenotyping, and genome and metagenome sequencing improvements allow a fast and relatively cost-effective recruitment of valuable information, which is currently the basis for understanding microbial diversity.

The new large-scale screening methods as MALDI-TOF MS using whole cell biomass in tandem with 16S rRNA gene sequencing allow a cheap and fast way to identify groups of organisms, putatively representing species. This approach may overcome the single strain species descriptions (SSSD), one of the major problems that taxonomy is facing. In addition and to my opinion, genome sequencing of at least the type strain of the new classified species should become a compulsory and routine requirement for any new description. As derived from the genome sequencing combined with the 16S rRNA gene sequence analysis it is possible to circumscribe most of the taxonomic categories. The submission of genome sequences in public repositories could equalize or (even better) substitute the type material required for any classification. In addition, there is a need to construct a protologue database that is interactive and searchable, linked to all metadata present in repositories, and generating a cumulative repository of taxonomic descriptions. Perhaps in the future these digitalized protologues may substitute the classical protologue texts embedded in the papers describing new taxa. Of course, phenotype needs to take again a major relevance in the description of new-cultured taxa. For this, there is a need to explore new methodologies as high-resolution mass spectrometry to substitute or complement some of the current practices that generally produce irrelevant information.

Finally, the important developments in microbial ecology, especially derived from metagenomic and single cell genomic practices, are generating genetic and genomic information that is of equivalent quality as for the cultured. If we do not want to face a divorce between taxonomy and molecular ecology, actions need to be taken, as (i) giving priority to the candidate taxa names and (ii) recognizing that the genome sequence could well become type material for a stable and universal classification.

FEMS7-3287
Bergey's Manual Trust: "Higher taxonomy of uncultivated microorganisms"

PROSPECTS AND CHALLENGES IN GENOME-BASED CLASSIFICATION OF UNCULTIVATED MICROORGANISMS

T. Long[1], G. Hughes[1], H. Lenwood[2], B. Vinatzer[1]
[1]Virginia Tech, PPWS, Blacksburg, USA
[2]Virginia Tech, Computer Science, Blacksburg, USA

Uncultivated microorganisms present challenges in regard to two core practices in bacterial taxonomy: polyphasic classification and the designation of species type strains. Therefore, uncultivated microorganisms can currently not be classified into validly named species. However, availability of genome sequences for many uncultivated microorganisms makes it possible to reconstruct genome-based phylogenies for uncultivated organisms as precisely as for cultivable organisms. Therefore, a genome phylogeny-based classification of uncultivated microorganisms is attainable. To do this in the absence of valid species names, we have used genome sequences to assign genome similarity-based Life Identification Numbers (LINs) to individual uncultivated microorganisms and to groups of closely related uncultivated microorganisms. LIN assignment was done using a prototype genome database, called LINbase, which efficiently identifies the genome in the database that is most similar to a newly up-loaded genome, calculates the average nucleotide identity (ANI) between the two genomes, and assigns a LIN based on the calculated ANI value. LINbase also provides an intuitive interface to describe any group of related organisms using any kind of metadata and to name these groups with either a validly published Latin binomial, if they represent a species, or in the case of uncultivated organisms, with any user-chosen non-Latin name. Therefore, LINbase offers an easy to use alternative for precisely classifying uncultivated microorganisms based on genome similarity. A demonstration of how to use LINbase will be included in the presentation.

FEMS7-3291
European Culture Collections' Organisation (ECCO): mBRCs: the new vision for culture collections

MIRRI: CONCATENATING CULTURE COLLECTIONS VIA THE EUROPEAN RESEARCH INFRASTRUCTURE

R. Aznar[1]
[1]*Universidad de Valencia, Colección Española de Cultivos Tipo, Paterna, Spain*

MIRRI (www.mirri.org), the 'Microbial Resource Research Infrastructure' is one of the projects included in the 'European Strategy Forum on Research Infrastructures' (ESFRI) 2016 roadmap. It originates from European Culture Collections joint work, from the 80's, to become microbial domain Biological Resource Centres (mBRC) in agreement with the OECD definition and will culminate in the MIRRI launch.

The MIRRI EU funding period ended in April 2016. Negotiations at governmental level are going to decide on the CCU location and to conclude legal issues. Currently, 6 countries have signed the MIRRI MoU supporting the establishment of an ERIC legal structure and up to eight more have taken part on the meetings held with national stakeholders.

Through MIRRI, European mBRCs have strengthened their links and collaborate in different initiatives such as the preparation of the new biotechnology standard (ISO/TC 276), the development of a common strategy for quality assurance across research infrastructures (within CORBEL), participate in the RItrain Horizon2020 project which envisages the development of a new generation of executives of national and international RIs. In addition, they share knowledge concerning the implementation of the Nagoya Protocol, for example producing the MIRRI Access and Benefit Sharing Manual (DOI: https://doi.org/10.5281/zenodo.284881); they cooperate to engage governments in supporting the operative phase of MIRRI.

At national level, mBRCs are key players in driving the MIRRI implementation process and at present many of them are working to create the nodes and/or the structures that will act as the focal points in each country to serve MIRRI coordinated activities, e.g. Laboratory networks in Portugal, Joint Research Units in Italy, Excellence Thematic Networks in Spain.

The spirit of MIRRI keeps alive despite the lack of funding and continues to influence the European life science landscape.

FEMS7-3295
European Culture Collections' Organisation (ECCO): mBRCs: the new vision for culture collections

IMPLEMENTING MICROBIOLOGY EDUCATION AND TRAINING IN DIGITAL ERA

N. Lima[1]
[1]*Centre of Biological Engineering - University of Minho, Centre of Biological Engineering, Braga, Portugal*

Culture collections have a long tradition in training people that directly, or indirectly, are interested in microbial taxonomy and in microbial preservation and management. Academic (PhD and Master), advanced as well as bespoke courses on related topics in this field are regarded of true added value for the educational and microbial research community in Europe. In addition, only with modern and appealing approaches we can reverse the strong decline in numbers of trained microbial taxonomists in Europe that has been observed over the last decades. Gaps in microbial resource management training and potential synergies have been identified and the establishment of an educational community to create a knowledge-based training network and implement lifelong educational and continuing professional development courses for people working within culture collections have been developed. Training courses involved both theoretical and wet laboratory experiences in microbiology not only covering elements of taxonomy and identification of microorganisms but also isolation, characterisation, preservation and use of microbial resources are of the importance. To support these actions e-learning materials for training activities and distance courses need to be implemented with innovative approaches. On top of this, MIRRI is involved on the RItrain (The Research Infrastructure Training Programme, http://ritrain.eu/) Horizon 2020 project. RITrain envisages improve and professionalize the training of managerial and leadership staff in research infrastructures (RIs). This is vital for the future success of Europe because access to excellent RIs underpins the success of today's research and innovation. The successful management and leadership of research infrastructure requires a complex collection of competencies, especially for those working across national borders. A flexible, modular executive master's degree is under development for RI managers and leaders, including executive directors of RIs, heads of finance and administration, heads of Human Resources and communication.

FEMS7-3298
European Culture Collections' Organisation (ECCO): mBRCs: the new vision for culture collections

NAGOYA PROTOCOL AND THE EU REGULATION ON ACCESS AND BENEFIT SHARING

G. Verkley[1]
[1]*Westerdijk Fungal Biodiversity Institute - KNAW, Collection, Utrecht, The Netherlands*

Nagoya Protocol and the EU regulation on Access and Benefit Sharing

Gerard J. M. Verkley

Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, the Netherlands

The Convention on Biological Diversity (CBD), which entered into force on December 29th, 1993, recognizes the sovereign rights of countries over their own biological resources, and aims at fair and equitable sharing of benefits arising from the utilization ('research and development") of genetic resources and associated traditional knowledge. Anyone who intends to access biological resources originating from areas within jurisdiction of a country that is a Party to the CBD, should first request for a Prior Informed Consent (PIC) and settle on Mutually Agreed Terms (MAT) with the competent authority in the country of origin, unless that Party has determined otherwise (for example, is giving free access). The Nagoya Protocol on "Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization" was adopted in Nagoya at the 10th Conference of the Parties of the CBD in 2010 and entered into force on October 12th, 2014. This legally binding treaty implements the CBD's third objective concerning the fair and equitable sharing of benefits arising from the utilization of genetic resources and associated traditional knowledge.

Regulation (EU) 511/2014 implements the Nagoya Protocol in the European Union. It applies from October 12th, 2014, the same day the Nagoya Protocol entered into force for the Union. To support the user community in its efforts to reach ABS compliance, the EC published a 'horizontal' guidance document (Guidance on the EU ABS Regulation implementing the Nagoya Protocol - Guidance on the scope of application and core obligations), to be followed soon by sectorial guidance documents.

MIRRI developed a Best Practice Manual to help microbial Biological Resource Centres (mBRC) to implement their ABS institutional policies with regard to genetic resources and associated traditional knowledge, and working procedures for the deposit of material in the public collection, the supply of cultures to third parties, and the delivery of other services. It also aims to increase transparency on how the mBRCs themselves conduct research on their holdings and lawfully utilize the genetic resources and associated traditional knowledge. This best practice manual was primarily designed for the management of collections of living microbial strains and their derivatives (e.g., DNA samples), but could be useful for all who receive microorganisms, use and supply them to colleagues or others outside their institutions for further use.

FEMS7-3329
From research to startup creation

INDUSTRIAL SECRET VERSUS PATENT PROTECTION IN BIOTECHNOLOGY

A. Diez Diaz[1]
[1]*PONSIP, Technology Transfer, Valencia, Spain*

In order to provide a wide review of the possibilities and best practices to bring ideas to market I will cover all the aspects related from the initial idea, to key concepts of market research, to review and analysis of Patent databases, concepts on funding, IP strategy, legal aspects of the development of the project, development of the prototypes, networking and sharing information drafting the business plan and landing in the market.

I will pay special attention to how to manage the intangible assets of the invention, under the topic of industrial secret versus patent protection in biotechnology

During the process I will provide tools to answer a number of questions like:

Is my idea a new idea?

What if the idea is just an idea?

Will someone steal the idea?

Will anyone buy the invention?

How will my invention fill the market?

Founding a company in the field of life sciences is associated with high initial investments for proper equipment and also capable minds are required. Scientists who dream of founding a company will find soon find how fierce the competition for ideas is.

Nowadays also investors demand much higher standards of technological validation for start-up ideas. In order to keep their own risk low and only come on board when the prospects of a profit are appropriate and well defined.

FEMS7-3316
From research to startup creation

PATENT PROTECTION IN MICROBIAL BIOTECHNOLOGY

J. Sherkow[1]
[1]*New York Law School, Innovation Center for Law and Technology, New York, USA*

CRISPR is a revolutionary advance in biotechnology: it allows molecular biologists to edit cells' DNA with ease and precision unimaginable even a decade ago. The technology is also the subject of contentious patent dispute between several universities, and governed by a maze licensing agreements among research institutions, nonprofits, biotech startups, and large pharmaceutical developers. Patents in the CRISPR space illuminate numerous problems—and advantages—of university-based intellectual property for groundbreaking technologies. The CRISPR patents herald the beginning of skepticism over interinstitutional collaboration, especially for lucrative "translational" technologies. And they have encouraged universities—otherwise committed to licensing their patents widely—to invest in for-profit surrogate companies to narrowly manage their license agreements for them. At the same time, CRISPR patents have allowed publicly minded research institutions to retain control over the technology to essentially prevent some of the technology's greatest potential abuses: runaway genetic modifications in the wild, also known as "gene drives"; seed-saving restrictions for agriculture; and germ-line human engineering. This talk will present an overview of these issues and discuss their application to future applications of CRISPR and other significant university-developed technology.

FEMS7-3309
ICSP Session: Role of genome sequence analysis in polyphasic taxonomy and prokaryotic systematic

Do we need the phenotype in the study of the systematics of Acinetobacter?

L. Dijkshoorn[1]
[1]*Department Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands*

Bacteria of the genus Acinetobacter are widespread in nature and have a complex taxonomic history. In the late 1980s, DNA-DNA hybridization (DDH) studies allowed for recognition of 20 DDH groups within the genus, seven of which were given valid species names. Since the early 2000s, the number of validly named species has risen from 7 to 54 (http://apps.szu.cz/anemec/Classification.pdf). Thirteen novel species descriptions are based on one strain only. Several novel species names are later heterotypic synonyms of previously described species. Furthermore, the genus comprises several groups of closely related species ('complexes'), members of which are difficult to distinguish phenotypically.
Novel species are, as a rule, identified using a polyphasic approach, i.e. a combination of phenotypic and genotypic methods. Recent methods for Acinetobacter include genomics (ANI), rpoB sequence analysis, mass spectrometry, analysis of fatty acids (and/or other chemotaxonomic markers), and metabolic and physiological characterization. The latter is based on a panel of ca. 40 in-house growth tests, which is more or less a standard requirement. This system is difficult to perform and no single test can be used to delimit the species. Chemotaxonomic markers have been used in a number of studies, but not systematically; markers vary and there are no libraries to compare results to. Altogether, despite the required phenotypic description in the proposal of novel species, this description does not seem to have great impact on the taxonomy of Acinetobacter. The question is whether there are alternatives for the current phenotypic characterizations or whether they can be abandoned.

FEMS7-3297
ICSP Session: Role of genome sequence analysis in polyphasic taxonomy and prokaryotic systematic

POLYPHASIC TAXONOMY: STILL JUSTIFIED OR FORCE OF HABIT?

I. Sutcliffe[1]
[1]*Northumbria University, Applied Sciences, Newcastle upon Tyne, United Kingdom*

Microbial systematics provides an essential framework for the activities of all microbiologists (and other biologists) and is of increasing importance as we engage with the task of mapping microbial diversity, of which we may have only seen the tip of the iceberg. However, whilst 16S rRNA based-phylogenetic methods revolutionised microbial systematics in the late 20th century, over the past two decades the discipline has become rather conservative and formulaic, constrained by the straightjacket of the 'polyphasic' taxonomic approach to describing novel taxa (most commonly at the genus/species levels). In the same period, the advent of affordable whole genome sequencing and the definition of increasingly well-established thresholds for delineating taxa using various measures of overall genome relatedness has the potential to shepherd in an era of genome-based systematics. This should circumvent the problem that many of the phenotypic and chemotaxonomic tests commonly performed as part of the 'polyphasic' taxonomic approach are of questionable value (including poor resolution, reproducibility and a lack of specialist expertise), particularly as descriptions of taxa based on single strains remain the norm. Thus the necessity for continuing with many routine 'polyphasic' methods can be strongly questioned. Nevertheless, there appears to be some reluctance on behalf of the microbial systematics community adopt genomics-based approaches and also other powerful new technologies such as MALDI-MS based typing. New initiatives, including the likely introduction of new publication formats and databases, are thus needed in order to sustain systematics as an attractive career choice for 21st century microbiologists.

FEMS7-3302
ICSP Session: Role of genome sequence analysis in polyphasic taxonomy and prokaryotic systematic

PHENOTYPIC CHECKLISTS: WHAT ARE THE ALTERNATIVES?

S. Venter[1], K. Maier[1], M. Palmer[1], J. Avontuur[1], W.Y. Chan[1], E. Steenkamp[1]
[1]University of Pretoria, Microbiology and Plant Pathology, Pretoria, South Africa

For many years bacterial taxonomist had to rely on phenotypic differences to characterize and identify species as it provided a measurable set of traits that could be used to distinguish among taxa. Over time this has caused strong dependence on a few commercial phenotyping systems, which seldom provide meaningful information on the biology of the bacterium. However, with the availability of whole genome sequences, bacterial diagnostics no longer needs to rely on this data. Apart from phylogenomics studies, genome data also provide the opportunity to deduct biologically relevant phenotype of the bacteria. Currently these predictions are based either on the construction of metabolic networks or by using specific trait predictor software. In this study, two such approaches were evaluated using a potential novel species closely related to *Bradyrhizobium pachyrhizi*. Although a number of common traits could be predicted and several shared and unique metabolic pathways were detected, a large part of these genomes could still not be linked to any function. The trait predictor software used is also limited in its scope and biological relevance. The focus of future research should be on improving the functional annotation of hypothetical proteins. These annotations will be crucial for the development of comprehensive genotype-phenotype association databases, which can be used for the predicting phenotypes and traits informative of the biology of the bacterial species under investigation. The focus of the phenotypic data collected should therefore shift from diagnostics-centered data to the characterization of the biology of the organism.

FEMS7-3279
Scientific publication explained: How to increase (or decrease) your chances of rejection

NAVIGATING THE PUBLICATION FOREST AND MAKING EDITORS AND REVIEWERS HAPPY

P. Bavoil[1], *M. Pacey*[2]
[1]*University of Maryland, School of Dentistry, Baltimore, USA*
[2]*Oxford University Press, FEMS Journals, Oxford, United Kingdom*

FEMS publishes five internationally recognised, peer-reviewed microbiology journals, all of which will be represented at the Congress and in the scientific programme. This workshop exploits the expertise of the editorial and publishing staff of the FEMS journals to take attendees from the preparation of research papers through to publication.

Through presentations, and a question and answer session with FEMS journals Editors-in-Chief, you will understand:

• what editors, reviewers and readers are looking for from authors
• how to organise and structure a paper to optimise presentation of your research
• the online submission process• the peer review process
• how to deal with comments from editors and reviewers
• what happens to a paper after acceptance for publication
• how to choose the best journal for you and your research
• open access requirements
• modern features available from the leading journals.

All may attend, but the workshop is particularly relevant to postgraduate, postdoctoral and early career researchers who wish to learn more about the publishing process.

FEMS7-2779
SEM-FEMS-ASM: Omics impact and perspectives on the microbial taxonomy, diversity and ecology

GENOME INFORMATION PROVIDES OBJECTIVE DATA FOR THE DESCRIPTION OF NEW BACTERIAL SPECIES, SO WHAT CAN GO WRONG?

A. Pérez-Cataluña[1]
[1]*University Rovira i Virgili, Basic Health Sciences, Reus, Spain*

Backgrounds

The number of bacteria genomes has increased exponentially in recent years and new tools for defining species have been developed. However, old and classical problems still remain i.e. existence of mislabelled genomes, which impact the results of any comparison. Other problems are associated to the fact that many are draft genomes of different qualities.

Objectives

To analyse these problems using our experience with the genus *Aeromonas* and *Arcobacter.*

Methods

We have analyzed deposited genomes of *Aeromonas* and *Arcobacter* from databases and re-evaluate their identity using ANI, *is*DDH and multilocus phylogenetic analysis (MLPA). Sequences of several housekeeping genes (*atpA*, *gyrA*, *gyrB*, etc), and of the 16S rRNA genes were retrieved from the genomes to construct trees that included the same genes for all the type strains.

Conclusions

Using these tools we have discovered that 36% of the studied *Aeromonas* genomes were mislabelled and genomes deposited as *Arcobacter* sp. could be assigned to known species. Considering these findings we recommended verifying the correct identity of a genome sequence using ANI, *is*DDH or MLPA before it is submitted to the database or used for any comparison. In addition 16S rRNA gene sequences available on those genomes were sometimes incomplete. Genomes labelled with incorrect names lead other authors to incorrectly suggest lowering the well-established ANI cut-off value (>96%) for the genus *Aeromonas* to 94-95%. So sequences and genomes are objective data, however, errors can still arise by a wrong interpretation of results.

FEMS7-2762
SEM-FEMS-ASM: Omics impact and perspectives on the microbial taxonomy, diversity and ecology

MINING THE ENVIRONMENT FOR NEW EUKARYOTIC DIVERSITY

R. Massana[1]
[1]*Institut de Ciències del Mar, Marine Biology and Oceanography, Barcelona, Spain*

Backgrounds

Microbial eukaryotes are integral members of marine ecosystems in terms of cell abundance, biomass, activity, and diversity and they play crucial roles in food webs and biogeochemical cycles. They are difficult to grow in culture and often cannot be identified by regular microscopy, so a variety of molecular tools have been applied to study their diversity.

Objectives

The objective is to identify the main microeukaryotic players in marine planktonic ecosystems and to determine the ecological performance of novel lineages.

Methods

Microeukaryotic diversity is first described by sequencing a phylogenetic marker gene from mixed assemblages, and then supported by PCR-free metagenomes. Specific probes are designed to target novel lineages by FISH (Fluorescent In situ Hybridization). Single cell genomics is used to access the genomes of uncultured cells.

Conclusions

Environmental molecular surveys have unveiled a large phylogenetic diversity and the presence of novel diversity. In this talk I will focus on a set of novel lineages within the stramenopiles that account for a significant fraction of the molecular signal, the MAST lineages. The abundance, distribution and genetic structure of the main MAST groups in the marine environment will be presented using specific probes for direct observation by FISH and parallel sequencing surveys using community DNA. MAST cells are also well represented in a collection of SAGs (Single Amplified Genomes) prepared from open sea surface samples, and preliminary genomic data for these uncultured cells will be presented. Certainly, the ecological and evolutionary significance of novel protist diversity is remarkable.

FEMS7-2705
SEM-FEMS-ASM: Omics impact and perspectives on the microbial taxonomy, diversity and ecology

GENOMIC INSIGHTS INTO SPECIES AND TRANSLATION OF THIS INSIGHT FOR ASSESSING SOIL MICROBIOMES

J. Tiedje[1]
[1]*Michigan State University, Center for Microbial Ecology, East Lansing, USA*

Backgrounds

Use genomic information for species resolution has gained traction over the last decade with the development and use of average nucleotide identity (ANI) and average amino acid identity (AAI) as well as use of other generally common protein coding genes. This greatly helps the resolution of taxa below the genus and especially the species level, which is the level necessary for ecologically meaningful assessment.

Objectives

The next stages needed are to use genomic information to provide some insight into ecological functions, and to resolve communities at the ecotype level, in effect narrowing the gap between sequence and ecological outcomes. To enable this, methodology is needed to assess species and ecotypes in complex communities with soil arguably the most complex at a small scale.

Methods

I will show examples of evaluating functions along a gradient of increasing genetic differences with agenus, of using the environment to reveal functional differences that reflect specialized physiologies, of characterizing diversity at a small environmental scale, and of using metagenomic sequence to assess diversity and composition at the ecofunctional and marker gene levels, and to quantify those components in communities.

Conclusions

This information provides deeper insight into the taxonomic composition to species and ecotype levels, spatial patterns and functional capabilities of soil and rhizosphere microbiomes.

FEMS7-3310
SEM-FEMS-ASM: Omics impact and perspectives on the microbial taxonomy, diversity and ecology

MINING MICROBIAL COMMUNITIES: ARE MULTI-OMICS ENOUGH?
C. Vilanova[1]
[1]*Darwin Bioprospecting Excellence SL, Biotechnology, Paterna, Spain*

TBA

FEMS7-3283
Special Event: Education: Engage your public! - #scicomm session and competition for young researchers

SCIENCE COMMUNICATION: GUIDELINES FOR YOUNG SCIENTISTS
L. Bowater[1]
[1]*university of East Anglia, Norwich Medical School, Norwich, United Kingdom*

Science Communication: seeking success and selling your story.

Science communication is at its heart, an opportunity to tell a story that will capture the attention of citizens and societies. But to be a successful storyteller it is important to use the language of your audience and not the scientific dialect and prose that most research scientists have been trained to read, write and orate. This session will draw on the personal experiences of the speaker in order to provide a short guide, specifically aimed at early career scientists, which outlines some of the tips and tricks you can use to enhance the way that you communicate science in general as well as your own personal research to a wider audience.

FEMS7-2913
AMR, public awareness, policies

AMR STRATEGY – FUTURE CHALLENGES FOR POLICYMAKING

E. Smith[1]
[1]*RAND Europe, Innovation- Health and Science, Cambridge, United Kingdom*

Antimicrobial resistance (AMR) is a public health challenge of global importance, as exemplified most recently by the UN Political Declaration on AMR. The UN Declaration demonstrates the recognition of AMR as a serious global threat by national governments and expresses unified political commitment to tackle the challenge. It also represents a remarkable result of sustained efforts to raise the profile of AMR and communicate the scale of the challenge to a wide global audience. Policy initiatives at many levels are now underway to tackle the issue at national, regional and international levels spanning the areas of new drug development, stewardship of existing antimicrobials and surveillance of their use. My talk explores the approaches being taken to address AMR in different policy contexts.

FEMS7-3315
AMR, public awareness, policies

PRIORITIES IN OUR COMBAT OF ANTIMICROBIAL RESISTANCE
J. Prof Dr[1]
[1]*Radboudumc, Medicine, Nijmegen, The Netherlands*

PRIORITIES IN OUR COMBAT OF ANTIMICROBIAL RESISTANCE

Jos W.M. van der Meer

European Academies Science Advisory Council (EASAC)

and Radboud University Medical Centre, Nijmegen, The Netherlands

The discovery and development of antimicrobial drugs during the past three quarters of a century has been one of the major advances in medical sciences. Not only did these drugs contribute to the combat of prevalent infectious diseases, but they also allowed great progress in many other areas of medicine (such as intensive care medicine, sophisticated surgery and cancer chemotherapy).

Over the past decade, the resistance of microorganisms (especially bacteria) to antimicrobial drugs has dramatically increased and reached crisis proportions. The major causes of this are twofold. First of all, the indiscriminate use of antimicrobial drugs in humans, animals and agriculture has led to immense selection pressure on microorganisms, leading to widespread resistance.

Secondly, the development of new antimicrobial drugs is stagnant since the 1980s. The current situation is that worldwide many bacterial infections are not treatable anymore.

Solutions to tackle this crisis are essentially:1. Implementation of restrictive and prudent use of antimicrobial drugs in the areas mentioned above (antibiotic stewardship).
2. Development of new antimicrobial drugs and treatment strategies.

The European Academies Science Advisory Council (EASAC) has called for attention to this problem since 2007. In 2014, an EASAC workshop identified the major obstacles in drug development, and in 2016, EASAC, together with the Federation of Academies of Medicine (FEAM), amended the recommendations formulated by O'Neill and proposed the priorities for Europe.

Although many organisations in the world (including UN and WHO) are intensively involved, it is clear that only with a number of breakthroughs the current crisis can be tackled.

FEMS7-2626
Antibiotics, novel approaches

DEVELOPING NOVEL ANTIMICROBIALS AGAINST GRAM-NEGATIVE PATHOGENS

O. Kuipers[1], M. Montalban-Lopez[2], J. Deng[1], Q. Li[1], A. Buivydas[1]
[1]University of Groningen, Molecular Genetics, Groningen, The Netherlands
[2]Faculty of Sciences University of Granada, Department of Microbiology, Granada, Spain

Developing novel antimicrobials against Gram-negative pathogens

Oscar P. Kuipers, Li Qian, Jingjing Deng, Andrius Buivydas, Manuel Montalbán-López

Department of Molecular Genetics, University of Groningen; o.p.kuipers@rug.nl

Antibiotic resistance in human pathogens is on the rise and this increase is not met with new approved antibiotics to combat these resistant bacteria. To solve this growing problem of resistance, alternative sources of antibiotics should be explored. One of these sources could be the class of ribosomally synthesized, post-translationally modified peptides called lantibiotics. Lantibiotics are well studied peptides that are stabilized by their characteristic rings formed with lanthionine or methyllanthione residues out of dehydrated Ser- and Thr-residues. We'll describe two different approaches to develop novel antimicrobials:

1. Biomodules for introducing various types of circular and heterocyclic modifications in lantibiotics.
2. Apply outer membrane perturbing agents to potentiate lantibiotic variants against Gram-negative pathogens.

FEMS7-0075
Antibiotics, novel approaches

ANTIBIOTICS FOR KILLING PERSISTER CELLS

K. Lewis[1]
[1]*Northeastern University, MA, USA*

We are experiencing an antibiotic crisis – our ability to discover novel compounds has diminished, and pathogens acquire and spread resistance largely unchecked. In chronic infections, the problem is exacerbated by the presence of dormant persister cells tolerant to killing by all antibiotics. As a result, chronic osteomyelitis or infections of patients with cystic fibrosis can be untreatable. In *E. coli*, stochastic expression of toxin/antitoxin modules contributes to persister formation. In *S. aureus*, we find that persisters are cells with a low level of ATP. This finding prompted us to revisit persister formation in *E. coli*, where we find a similar mechanism. A decrease in ATP is a general, and probably main, mechanism of persister formation in bacteria. A decrease in ATP provides a satisfactory explanation for the presence of drug tolerant persisters. Currently available bactericidal antibiotics kill by corrupting ATP-dependent targets such as protein or peptidoglycan synthesis. When ATP is low, targets are inactive, and cells become tolerant to killing. We identified two compounds that kill persisters without a requirement for ATP. Acyldepsipeptide dysregulates the Clip protease, forcing the cell to self-digest. Lassomycin, which we discovered from a screen of uncultured bacteria, activates the ClpC1 chaperone of the mycobacterial Clp protease, forcing it to digest ATP. We also developed a general approach to eradicate persisters with pulse-dosing of antibiotics. Teixobactin, an antimicrobial essentially free of resistance development, eradicates persisters when periodically applied to a culture of *S. aureus* in which persisters are allowed to resuscitate after application of the antibiotic.

FEMS7-0538
Antibiotics, novel approaches

INNOVATIVE ANTIBIOTICS FROM MICROBES: IDENTIFICATION, MODE OF ACTION AND RESISTANCE MECHANISMS

R. Müller[1]
[1]*Helmholtz Institute for Pharmaceutical Research Saarland, Microbial Natural Products, Saarbrücken, Germany*

Microorganisms are accepted as a valuable source for chemical biology tools and drug leads. An outstanding group of microorganisms is represented by the ubiquitous myxobacteria, a largely underexploited resource for natural products (NP). Analyzing the few myxobacterial genomes known to date leads to the conclusion that they contain up to 50 respective biosynthetic gene loci per strain indicating the enormous genomic potential for the production of NPs. However, only few compounds are typically known per microbial isolate. In a comprehensive MS-based study we have addressed the question of compound diversity in correlation with phylogenetic diversity and found clear and measurable evidence that new compounds are most likely to be found in currently uncharacterized microbial genera and families.

Once novel microbial NPs are identified, their potential as antibiotic drug leads is analyzed by defining pharmaceutical properties and potential resistance mechanisms of pathogens against the compound. Target identification, which is usually a complex and rather unpredictable biochemical research endeavour, has become essential for drug development for numerous reasons including the possibility to rationally optimize lead compounds based on their molecular structure-target-complex. During the last decades genomics has become an integral part of NP drug research and allows not only for directed approaches to discover new natural products: Target identification might be achieved by studying self-resistance mechanisms within the producer strains or, alternatively, by defining the molecular basis of resistance in pathogens by whole genome sequencing of evolved bacterial resistance.

The presentation will cover some examples of novel antibiotics and target identification of microbial NPs.

FEMS7-3311
Bacterial persistence and toxin - antitoxins

TOXIN-ANTITOXIN SYTEMS AND THEIR CHAPERONES

G. Pierre[1]
[1]*CNRS Toulouse University, LMGM/CBI, Toulouse, France*

Ambre SALA, Patricia BORDES, Sara AYALA, Pauline TEXIER, Nawel SLAMA, Anne-Marie CIRINESI, Valérie GUILLET, Lionel MOUREY, Samuel TRANIER, Marie-Pierre CASTANIE-CORNET and Pierre GENEVAUX

Abstract Bacterial type II toxin-antitoxin (TA) systems are small genetic modules typically composed of a toxin and a more labile cognate antitoxin, which binds and inhibits the toxin. In response to certain stress the antitoxin is rapidly degraded by proteases and the free active toxin generally targets essential cellular processes, leading to a reversible growth inhibition known to facilitate bacterial persistence to drugs, virulence and survival in response to environmental insults. *Mycobacterium tuberculosis*, the causing agent of human tuberculosis, encodes 79 putative toxin-antitoxin systems and it has been proposed that persistence induced by active toxins might be relevant for its pathogenesis. Tripartite TAC modules are atypical TA systems composed of a conserved two-component TA that is specifically controlled by a molecular chaperone related to the canonical SecB chaperone known to facilitate protein export in bacteria. In this case, the SecB-like chaperone interacts with the TAC antitoxin and protects it from both aggregation and degradation, and is thus strictly required for neutralization of the toxin by the antitoxin. How does the mycobacterial SecB-like chaperone control the toxin activation cascade, and to what extent such activation is important for *M. tuberculosis* persistence and virulence are so far unresolved questions. Herein we investigate the molecular mechanism by which classical TA systems can turn into chaperone-addicted modules as well as the specialization events that redirect a generic export chaperone towards the control of TA systems.

FEMS7-1736
Bacterial persistence and toxin - antitoxins

BACTERIAL MULTIDRUG TOLERANCE, MAGIC SPOT AND TOXIN – ANTITOXINS

S. Semsey[1], *A. Harms*[1], *K. Syal*[1]
[1]*University of Copenhagen, Department of Biology, Copenhagen N, Denmark*

Bacterial persister cells evade antibiotic-induced killing by entering a physiologically slow-growing state and may cause antibiotic treatment failure and relapsing infections. Persister cells form both stochastically and in response to environmental cues, by various pathways that are usually controlled by the second messenger (p)ppGpp. Remarkably, both type I and type II toxin-antitoxin (TA) modules have been suggested to play significant roles in persister formation in several model systems. Thus, the type I TA module *hokB*/*sokB* of *Escherichia coli* K-12 induces persistence by a mechanism that depends on (p)ppGpp and the universally conserved Obg GTPase. In contrast, type II TA modules (e.g. *relBE*, *mazEF*, *tacAT* etc.) induce persistence via a pathway that depends on (p)ppGpp, Lon and polyphosphate, not only in *E. coli* but also in *Salmonella enterica*. More recently, it was shown that upregulation of drug efflux pumps (e.g. TolC) cells may also contribute to the formation of persisters. Thus, persister cells are heterogeneous, formed by multiple pathways that may be activated independently or in parallel. The latest developments in the persistence field will be discussed.

FEMS7-2613
Bacterial persistence and toxin - antitoxins

SALMONELLA FORMS INTRACELLULAR PERSISTERS WITH TACT

S. Helaine[1]
[1]*Imperial College London, CMBI, London, United Kingdom*

Persister bacteria are non-growing, antibiotic insensitive cells, the progeny of which are sensitive to antibiotics. Bacterial persistence is a common phenotype expressed by a large number of bacterial species and is thought to be responsible for relapsing infections. During *Salmonella* infection of macrophages an important proportion of bacteria enter a persister state via the action of class II toxin-antitoxin modules. These toxin-antitoxin modules encode a stable toxin that inhibits a vital cellular process and a labile, neutralising antitoxin, which is degraded under conditions of stress but otherwise binds and inactivates the toxin. We investigate the activity of three of these toxins, which are acetyltransferases and how bacteria recover from the persistent state.

FEMS7-0082
Biofilms, formation and persistance

TOWARDS KNOWING THE ENEMY: INVESTIGATION OF BACTERIAL PERSISTER PHYSIOLOGY

M.P. Brynildsen[1]
[1], *USA*

Bacterial persisters are phenotypic variants with extraordinary tolerances toward antibiotics. Persister survival during antibiotic stress has been attributed to inhibition of essential cell processes, which prevents antibiotics from corrupting their primary targets, followed by reversion to normal physiology upon removal of the antibiotic. In recent years, many studies have shed light on the genetic basis of persistence by identifying genes whose presence, absence, or abundance alter the levels of persisters in bacterial populations. However, far less is known about the physiology of persisters and how that confers survival under antibiotic stress. For example, little is known about the response of persisters to antibiotics, although the phenotype is defined based on the outcomes of antibiotic treatments. Lack of growth is a phenotypic quality that increases the likelihood bacteria would be persisters; however, most non-growing cells succumb to antibiotic treatments and it remains ill-defined what other phenotypic qualities differentiate persisters from non-persisters in growth-inhibited populations. In this talk, I will discuss my group's recent work to characterize persister physiology in order to gain a more mechanistic understanding of how persisters survive in the face of certain death.

FEMS7-0539
Biofilms, formation and persistance

ROLE OF PHYSIOLOGICAL ADAPTATION IN BIOFILM RESISTANCE AND VIRULENCE

T. Coenye[1]
[1]*Ghent University, Gent, Belgium*

The susceptibility of microbial biofilms towards antimicrobial agents is mediated in part by changes in physiology and metabolism. In this presentation I will provide an overview of our recent work on this topic, with a focus on *Burkholderia cenocepacia* and *Staphylococcus aureus*. Items that will be discussed include the role of reactive oxygen species (ROS) in antibiotic-mediated killing, the effect of metabolic adaptation to the presence of other species on susceptibility of multispecies biofilms, and the role of small non-coding RNAs and mini-proteins on physiology and susceptibility of sessile *B. cenocepacia* cells.

FEMS7-1638
Biofilms, formation and persistance

DYNAMICS OF RESPONSIVENESS OF BIOFILM BACTERIA

S. Haeussler[1], M. Muesken[1]
[1]Helmholtz Centre for Infection Research, Molecular Bacteriology, Braunschweig, Germany

DYNAMICS OF RESPONSIVENESS OF BIOFILM BACTERIA

Many efforts to decrease the burden of chronic infections have been made; however in spite of the initial enthusiasm and the huge literature on the identification of novel anti-biofilm compounds, to date no antimicrobials are in clinical use to specifically treat biofilm infections. An alternative option to the search for new anti-biofilm compounds is to concentrate on enhancing the activity of today´s antibiotics. Antibiotics are *very effective* in the *treatment* of bacterial infections. However, their use in the successful eradication of biofilm-associated infection relies on our ability to overcome two main problems. First, there is a need for knowledge on the resistance profile of the individual infecting bacterial isolate under biofilm-growth conditions. The replacement of susceptibility testing of planktonic bacteria by biofilm-specific resistance profiling in individual bacterial isolates might allow for a targeted anti-biofilm therapy. In addition, new treatment strategies that promote antimicrobial efficacy of antibiotics in clinical use will have to be developed to overcome the second limitation of current treatment, which is the incomplete killing of the biofilm population. New treatment regimens need to be adapted to accommodate killing of high density biofilm-associated infections and which avoid the Sisyphean fate of fast killing and reconstituting regrowth of bacterial biofilm populations.

FEMS7-3318
Biofilms, formation and persistance

IDENTIFYING GENES UNDER ADAPTIVE EVOLUTION DURING BACTERIAL BIOFILM DEVELOPMENT

J. Webb[1]
[1]*University of Southampton, Biological Sciences, Southampton, United Kingdom*

Title: Identifying genes under adaptive evolution during bacterial biofilm development

Growth of bacteria within biofilms can quickly lead to within-population genetic diversity; but the generation of this diversity and the dynamics of evolution with bacterial biofilms remain poorly understood. By using genomic approaches, we have examined genetic diversification that occurs during biofilm formation of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. For both organisms, we discovered extensive parallel evolution between biological replicates. For *P. aeruginosa*, we used deep sequencing to carry out studies of within-population genetic diversification occurring during biofilm formation. Parallel evolution occurred between biological replicates, at the level of pathways, genes, and even individual nucleotides. For *S. pneumoniae,* whole genome sequencing of 12 colonies with a small-colony variant (SCV) phenotype, each from independent biofilm experiments, revealed that all SCVs studied had mutations within the DNA-directed RNA polymerase delta subunit (RpoE). These studies show that biofilms very generate very strong selection for certain mutations which is providing new understanding of genes that are central to biofilm development.

FEMS7-0048
Evolution and genome plasticity

KILLING FOR NEW GENES - HOW VIBRIO CHOLERAE STEALS DNA FROM NEIGHBORING BACTERIA

M. Blokesch[1]
[1]*Swiss Federal Institute of Technology Lausanne EPFL, School of Life Sciences, Lausanne, Switzerland*

Killing for new genes - how *Vibrio cholerae* steals DNA from neighboring bacteria

Vibrio cholerae, the causative agent of cholera, is considered to be an important model organism for studying infectious diseases. However, compared to its pathogenic potential in humans, much less is known about the bacterium's lifestyle in its primary habitat, the aquatic environment. Such environmental habitats often contribute to pathogen emergence, which is frequently accomplished through the acquisition of novel genetic information by means of horizontal gene transfer (HGT). Natural competence for transformation as a mode of HGT plays a key role in bacterial evolution and *V. cholerae* enters the competence state upon growth on chitinous surfaces.

In this talk, I will give a brief summary about the regulatory network that drives competence in *V. cholerae*. I will also show genetic and imaging-based data that illustrate that the type VI secretion system (T6SS) of diverse pandemic *V. cholerae* strains is part of the competence regulon. T6SS is a molecular killing device, and it fosters HGT by the deliberate killing of neighboring bacteria followed by the absorption of their DNA. The extent of such DNA transfer events has not been investigated in the past. We have addressed this lack of knowledge through the analysis of transformants. These data provide evidence for the transfer of chromosomal stretches of significant length. I will conclude my presentation with the hypothesis that competence-induced kin-discriminated neighbor predation linked to DNA uptake might be a common biological theme.

FEMS7-1951
Evolution and genome plasticity

EVOLUTION OF BACTERIA COLONIZING THE INTESTINAL TRACT: MUTATION VERSUS HORIZONTAL GENE TRANSFER EVENTS AS DRIVERS OF ADAPTATION

I. Gordo[1], *N. Frazão*[1]
[1]*Instituto Gulbenkian de Ciência, Evolutionary Biology, Oeiras, Portugal*

The contribution of mutation relative to horizontal gene transfer (HGT) to the evolution of bacteria inhabiting the gut microbiota is currently unknown. Here we use an *Escherichia coli* strain colonizing specific pathogen free mice to unravel the relative role of these two mechanisms for the emergence of strain diversity within the microbiota of the mammalian intestine. Through experimental evolution, next generation sequencing, phenotypic assays and microscopy we reveal the rapid occurrence of multiple HGT events, mediated by phages from the resident microbiota, to the colonizing *E. coli*. We further show that the ecological context in which colonization with a new lineage occurs is a key determinant of the mechanism of its future evolutionary change. We find that *E. coli* evolution is dominated by phage-mediated HGT when its direct competitors are present in the microbiota at high abundance, whereas otherwise it is dominated by mutation accumulation. Furthermore we demonstrate that the genome integration of incoming phages by the new *E. coli* colonizer results in immunity against phages produced by its resident competitors, allowing coexistence between phylogenetic groups A and B1 strains of *E. coli* in the mouse gut.

FEMS7-0510
Evolution and genome plasticity

SIGNATURES OF RECENT EMERGENCE IN BACTERIAL PATHOGENS

J. Parkhill[1]
[1], *United Kingdom*

Identifying the mode of transmission and acquisition of nosocomial pathogens is of crucial importance in designing effective surveillance and intervention for infection control in hospitals. We have used large-scale, systematic, longitudinal surveys of important multi-drug resistant (MDR) pathogens causing bloodstream infections in UK hospitals to understand the population structure of these organisms. Whole genome sequencing and phylogenetic reconstruction of these isolates allows us to accurately reconstruct the population history of these MDR pathogens across the UK and Ireland. These population histories demonstrate two clear modes of transmission: recent clonal spread within and between hospitals, and older, more diverse populations indicative of commensal spread within the wider population. Understanding these differences will enable tailored infection control procedures to minimise nosocomial acquisition of these important pathogens.

FEMS7-3312
Extreme environments, archaea, and bacteria

METABOLIC DIVERSITY OF THERMOPHILIC PROKARYOTES: NEW GROUPS, NEW PROCESSES

E. Bonch-Osmolovskaya[1]
[1]*Federal Research Center of Biotechnology RAS, Winogradsky Institute of Microbiology, Moscow, Russia*

Thermophilic microbial communities are actively studied during past three decades. However, the representatives of new phylogenetic and metabolic groups of thermophiles could still be isolated. Here we describe new lithoautotrophic and organotrophic bacteria from volcanic and subsurface thermal habitats.

Volcanic environments are rich in sulfur compounds which can be used by microorganisms in catabolic processes. We found that a phylogenetically diverse thermophilic lithoautotrophs of phyla *Thermodesulfobacteria* and *Deltaproteobacteria* are capable to grow by disproportionation of elemental sulfur, thiosulfate or sulfite. They belong to new genera *Thermosulfurimonas, Dissulfuribacter, Dissulfurimicrobium, Dissulfurirhabdus*, as well as to the previously known genus *Caldimicrobium* that was found to play a pivotal role in Kamchatka Peninsula (Russia) hot springs. Representatives of genera *Thermosulfurimonas* and *Dissulfuribacter* were also found to be able to couple elemental sulfur oxidation with reduction of nitrate to ammonia. Another new metabolic group of sulfur-metabolizing thermophilic lithoautotrophic bacteria is that of obligate hydrogen-oxidizing sulfite reducers represented by *Thermodesulfitimonas* gen. nov.

Thermophilic microorganisms capable to degrade polymeric substrates are, in majority, obligate anaerobes with fermentative metabolism. We found two phylogenetic groups of bacteria that can hydrolyze polymeric substrates in the course of anaerobic respiration. Thermophilic planctomycetes of genera *Thermogutta* and *Thermostilla* grow anaerobically on various polysaccharides reducing nitrate or elemental sulfur, while bacteria of genus *Melioribacter* (phylum *Ignavibacteria*) utilize cellulosic substrates in the course of nitrate, iron or arsenate respiration. Thus, the anaerobic process of complex organic substrates mineralization is performed by a single microorganism and not by a complex microbial community as it is usually assumed.

FEMS7-3314
Extreme environments, archaea, and bacteria

DISCOVERY OF CARBOHYDRATE ACTIVE ENZYMES FROM HYPERTHERMOPHILIC MICROORGANISMS: GENOME AND METAGENOME

M. Moracci[1], A. Strazzulli[1], R. Iacono[2], R. Giglio[2], L. Maurelli[2], B. Cobucci-Ponzano[2]
[1]University of Naples "Federico II", Department of Biology, Naples, Italy
[2]National Research Council, Institute of Biosciences and BioResources, Naples, Italy

Hyperthermophilic Bacteria, Archaea and viruses, populating marine and terrestrial hydrothermal sites, are of interest as a source of biocatalysts because of their uncommon resistance to heat and other protein denaturants, making them attracting tools for industrial applications. In particular, carbohydrate active enzymes (CAZymes) from these sources have recognized application for innovative bioprocesses as the saccharification step of the pretreatment of lignocellulose biomass for second generation biorefineries.

In the framework of the discovery of new CAZymes for biocatalysis and biotransformations for industrial applications, we identified novel glycosidases from sequenced genomes from (hyper)thermophiles and metagenomes obtained from hydrothermal vents. Their characterization and exploitation in enzymatic cocktails for the conversion of biomass from an energy crop will be discussed.

Acknowledgement

This work was supported by the projects PON01_01966 “ENERBIOCHEM” and PON03PE_00107 “BioPolis” of the Programma Operativo Nazionale Ricerca e Competitività 2007-2013 -MIUR-Italy and by the Short-term mobility Programme “STM 2013” of the National Research Council of Italy

FEMS7-2621
Extreme environments, archaea, and bacteria

METAGENOMICS OF HYPERSALINE ENVIRONMENTS: THE CASE OF SPIROBACTER

A. Ventosa[1], *M.J. León*[1], *B. Vera-Gargallo*[1], *C. Sánchez-Porro*[1]
[1]*University of Sevilla, Department of Microbiology and Parasitology, Sevilla, Spain*

Hypersaline environments are widely distributed on our planet. They are represented by aquatic as well as by saline terrestrial habitats. However, most microbiological studies have been focused on saline aquatic systems, which are classified as thalassohaline and athalassohaline habitats, depending on the relative proportions of salts, derived from their marine or non-marine origin, respectively.

Besides hypersaline lakes, the solar salterns-which are traditionally used for the commercial production of salt-are excellent models for studying the microbial diversity and the effect of salinity on the microbial populations. They are constituted by a series of ponds with increasing salinities, from seawater to salt saturation (designed as crystallizers). One of the salterns that has been extensively studied for the last 40 years is located in Santa Pola, Alicante (Spain), at the Mediterranean coast. These studies were based initially on culture-dependent techniques, lately on molecular approaches, and during recent years on metagenomic analyses. All these accumulated data have permitted to determine in detail the prokaryotic populations inhibiting the ponds of this saltern, and compare them with those of other salterns from different locations. As could be expected, the microbial diversity is drastically reduced with increasing salinity. At the crystallizer ponds the dominant prokaryotic groups are practically limited to Euryarchaeota, represented by members of the class Halobacteria (*Haloquadratum*, *Halorubrum*) as well as Nanohaloarchaea, and at a lower extent some Bacteroidetes (*Salinibacter*) representing the Bacteria. In contrast, the microbial population at intermediate salinity ponds is more diverse, represented by species belonging to several higher taxa (Euryarchaeota, Gammaproteobacteria, Bacteroidetes, Actinobacteria, Alphaproteobacteria, Verrucomicrobia, Firmicutes, etc.). Despite the enormous efforts in order to cultivate the representatives of these microbial groups, which has permitted the isolation of a large number of microbes representing new genera and species of Archaea and Bacteria, a large proportion of the microbial population present on these habitats has not yet been isolated in pure culture and characterized.

The analyses of the metagenomic databases derived from two concentrator ponds from Santa Pola saltern (with 19 and 13 % total salts) and one pond from another saltern located in Isla Cristina, Huelva (South Spain) with 21 % salts, have shown that a large percentage of metagenomic sequences were related to a new group of Gammaproteobacteria, closely related to the genus *Alkalilimnicola*, within the family *Ectothiorhodospiraceae*. Initially we were able to isolate a single strain of this new abundant bacterial group and named it as a new genus and species, *Spiribacter salinus*, on the basis of the peculiar morphology, being curved cells which at a later stage have a tendency to form long spiral cells. More recently, we have characterized additional strains which have been described as two new species of this genus: *Spiribacter curvatus* and *Spiribacter roseus*. Their complete genomes have been sequences and assembled into a single contig; they have genome sizes ranging from 1.7 to 1.9 Mb, showing the smallest genomes currently described for any member of the family *Ectothiorhodospiraceae*. The recruitment of their genomes with available metagenomic databases obtained from habitats with different salinities have permitted to determine that this new bacterial genus represents a group of species that are abundant on the intermediate salinity ponds (ca. 10 to 25 % salts) but are not found on marine habitats as well as at high salinities, i.e. the crystallizer ponds of the salterns. Our studies show that metagenomics, which enables to determine the microbial diversity of a natural environment, can help to generate information permitting the identification of previous unknown microbes and eventually their isolation in pure culture and further characterization.

FEMS7-3299
Fungal cell biology

WHAT DRIVES RHYTHMICITY? IDENTIFYING METABOLIC PROCESSES THAT DRIVE THE YEAST RESPIRATORY OSCILLATION

N. Hoyle[1], D. Tourigny[1], J. Robertson[2], R. Edgar[1], C. Frezza[3], S. da Costa[3], S.Y. Peak-Chew[1], J. O'Neill[1], H. Causton[4]
[1]MRC Laboratory for Molecular Biology, Cell Biology, Cambridge, United Kingdom
[2]Middle Tennesse State University, Department of Biology, Murfreesboro, USA
[3]MRC Cancer Unit, Cancer Unit, Cambridge, United Kingdom
[4]Columbia University Medical Center, Dept. of Pathology and Cell Biology, New York, USA

Yeast respiratory oscillations (YROs), are robust, short period rhythms that arise spontaneously when yeast are grown aerobically under nutrient-limited conditions in continuous culture. They are characterised by transient and intense bursts of respiration and act to coordinate much of cellular metabolism. YROs share diverse features with circadian rhythms including temperature compensation, cyclic changes in redox state, coupling with the cell division cycle and period determination by casein kinase I. This suggests they either represent an ancient and conserved phenomenon that is likely to be driven by mechanisms common to all aerobic eukaryotes, or draw on general metabolic functions, however, the underlying mechanism is not known.

We have taken a systems-wide approach to understanding how rhythmicity is mediated. We have generated a comprehensive description of metabolites, proteins and protein modifications in yeast undergoing oscillations of different periodicities and used it to derive a mechanistic model. Our data identify additional features shared between circadian rhythms and YROs, reveal key processes that promote the sequential occurrence of distinct metabolic processes and show how the cyclic remodelling of cellular function is underpinned by cycles of anabolism and catabolism.

FEMS7-0090
Fungal cell biology

CELL WALL ADHESINS GOVERN BIOFILM FORMATION IN THE PATHOGENIC YEASTS CANDIDA GLABRATA AND CANDIDA PARAPSILOSIS

J. Fernández[1], A. Moreno[1], E. Gómez[2], A.D. De Boer[1], H.L. Dekker[3], I. De la Pinta[4], O. Bader[2], G. Quindós[4], P.W.J. De Groot[5]
[1]University of Castilla-La Mancha, Molecular Mycology, Albacete, Spain
[2]University Medical Center Göttingen, Institute for Medical Microbiology, Göttingen, Germany
[3]University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, The Netherlands
[4]Universidad del País Vasco UPV/EHU,
Department of Inmunología- Microbiología y Parasitología- Facultad de Medicina y Odontología, Bilbao, Spain
[5]University of Castilla-La Mancha/Science & Technology Park Castilla–La Mancha, Molecular Mycology, Albacete, Spain

The yeast species *Candida glabrata* and *Candida parapsilosis* are, next to *Candida albicans*, among the most frequent fungal causes of mucosal and life-threatening bloodstream infections in immunocompromised persons. The cell walls of these *Candida* species plays an important role in the host-pathogen interactions that lead to the establishment of infections. Attachment to human host tissues or abiotic medical devices and subsequent biofilm formation represents the first phase in this process and is mediated by cell wall-localized adhesins. By analyzing the wall proteomes of hyper-adhesive clinical isolates using mass spectrometry and comparison to reference strains, we tested whether these isolates display differential and increased incorporation of adhesins. Wall proteome analysis identified a core proteome of about 20 proteins present in each strain under all analyzed conditions. In addition, proteomic analysis of hyper-adherent strains under biofilm-forming conditions showed (i) higher levels of adhesins that are also found in the reference strains and (ii) incorporation of novel adhesins that are not found in the reference strains. Thus, the hyperadhesive capacity of hyper-adherent *Candida* isolates seems to be correlated with increased and differential incorporation of cell wall adhesins. Current studies in our laboratory are aimed at performing functional and structural characterizations of the novel adhesins and at elucidating their role in primary host-pathogen interactions, antifungal drug resistance, and virulence.

FEMS7-3013
Fungal cell biology

AUTOPHAGY IN DEGRADATION OF PEROXISOMAL AND CYTOSOLIC ENZYMES OF METHANOL

A. Sibirny[1]
[1]*Institute of Cell Biology NAS of Ukraine, Molecular Genetics and Biotechnology, Lviv, Ukraine*

AUTHOPHAGY IS RESPONSIBLE FOR DEGRADATION OF CYTOSOLIC ENZYMES OF METHANOL METABOLISM IN THE YEAST *PICHIA PASTORIS*

Nina V. Bulbotka[1], Kateryna O. Levkiv[1], Olena V. Dmytruk[1] and Andriy A. Sibirny[1,2]

[1]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov Street, 14/16, Lviv 79005 Ukraine; [2]Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, Rzeszow 35-601 Poland

Background

Methanol metabolism in the methylotrophic yeast *Pichia pastoris* metabolizes both in peroxisomes (alcohol oxidase, catalase, dihydroxyacetone synthase) and cytosol (formaldehyde and formate dehydrogenase, fructose-1,6-bisphosphatase). The shift of methanol-grown cells to glucose initiates inactivation of both peroxisomal and cytosolic enzymes. The mechanisms of inactivation of cytosolic enzymes involved in methanol metabolism are not known. Inactivation and degradation start from glucose sensing. Specific glucose sensor has been identified in *P. pastoris*, however, its functional groups responsible for glucose recognition have not been identified.

Objectives

Objectives of this work were to study role of autophagy in inactivation of cytosolic enzymes of methanol metabolism in *P. pastoris*: formaldehyde and formate dehydrogenase and fructose-1,6-bisphosphatase. We also aimed in identification of the fragments of glucose sensor Gss1 required for glucose-induced inactivation of these enzymes.

Materials and Methods

Standard methods of yeast molecular genetics (vector construction, transformation) and biochemistry (western blotting, enzyme assays) were used.

Conclusions

During inactivation of fructose-1,6-bisphosphatase, degradation of the corresponding protein occurred and proteasomal inhibitor MG-132 only slightly inhibited this process. At the same time, degradation of fructose-1,6-bisphosphatase was defective in the mutants of *P. pastoris pep4 prb1* and *ccz1Δ, mon1Δ* or *ypt7Δ* defective in vacuolar proteinases suggesting the role of vacuoles in degradation of

cytosolic enzyme of methanol metabolism. Degradation of fructose-1,6-bisphosphatase was also strongly retarded in *gss1Δ* mutant defective in glucose sensor suggesting the role of glucose signaling in this process. Thus, glucose signaling and specific autophagic degradation apparently are involved both in degradation of both fructose-1,6-bisphosphatase and alcohol oxidase.

FEMS7-0043
Fungal cell biology

THE IDENTITY OF HYPHAE IN A FUNGAL MYCELIUM

H. Wosten[1]
[1], *The Netherlands*

Backgrounds

Liquid shaken cultures of fungi consist of populations of micro-colonies that differ in size and gene expression. For instance, large micro-colonies express other genes encoding secreted proteins than small micro-colonies. Heterogeneity is also observed between zones and within a zone of micro-colonies. The latter came to a surprise since fungi were assumed to share a common cytoplasm, in particular within a particular zone, due to large pores in the septa that compartmentalize hyphae.

Objectives

We studied the mechanisms underlying the paradox between heterogeneity and an assumed continuous cytoplasm, and its consequences for the role of individual hyphal compartments.

Methods

We used laser dissection combined with fluorescence microscopy and modeling.

Conclusions

The majority of septa of growing hyphae of *Aspergillus* and *Penicillium* are closed by Woronin bodies. Modelling showed that even open septa of these fungi act as a barrier for inter-compartmental cytoplasmic mixing. Growth speed of apical compartments was not affected when they were dissected from subapical compartments. These compartments started to branch when the apical compartment was damaged.

Apical compartments are self-providing units with subapical compartments functioning as a back-up system for branching when the apical compartment is damaged while exploring its substrate. The self-providing nature of compartments explain why hyphae within and between zones of a micro-colony are heterogeneous with respect to gene expression and molecular composition.

FEMS7-3325
Global microbiome

GENOME CENTRIC METAGENOMICS IN ENVIRONMENTAL MICROBIOLOGY

M. Albertsen[1]
[1]*Aalborg University, Center for Microbial Communities, Aalborg, Denmark*

Small subunit (SSU) ribosomal RNA (rRNA) genes have been the standard phylogenetic markers for the study of microbial evolution and diversity for decades. However, the essential reference databases of full-length rRNA gene sequences are underpopulated, ecosystem skewed, and subject to primer bias; which hampers our ability to study the true diversity. In this talk, I will present out latest method development that combines poly(A)-tailing and reverse transcription of SSU rRNA molecules with synthetic long-read sequencing, to generate millions of high quality, full-length SSU rRNA sequences without primer bias. We applied the approach to complex samples from seven different ecosystems and obtained more than 1,000,000 SSU rRNA sequences from all domains of life. The novel diversity is overwhelming and include several potentially new archaeal phyla of the deeply branching Asgard Archaea, which are previously suggested to bridge the gap between prokaryotes and eukaryotes. This approach will allow expansion of the rRNA reference databases by orders of magnitude and will enable a comprehensive census of the tree of life. With a fully populated SSU tree of life, it will be possible to prioritize efforts towards making a fully populated genome tree of life. To demonstrate the progress with these efforts, I will also discuss our recent progress on extraction of complete (closed) genomes from metagenomes using high-throughput long-read Nanopore sequencing.

FEMS7-0073
Insect-microbe interactions

IMMUNE RESPONSES INVOLVED IN ENDOSYMBIONT CONTROL AND HOST HOMEOSTASIS

A. Heddi[1]
[1]*INSA Lyon, BioSciences, Villeurbanne, France*

Many insects sustain long-term relationships with intracellular symbiotic bacteria that provide them with essential nutrients. Such endosymbiotic relationships have likely emerged from ancestral infections of the host by free-living bacteria, the genomes of which experience drastic gene losses and rearrangements during the host-symbiont coevolution. While it is well documented that endosymbiont genome shrinkage results in the loss of bacterial virulence genes, whether and how the host immune system evolves towards the tolerance and control of bacterial partners remains poorly understood.

Insect immune system is permanently struggling to keep beneficial symbionts while activating defense effectors to prevent pathogenic infections. We showed that weevils have selected a 'compartmentalization strategy' that consists in secluding endosymbionts within specialized host cells, the bacteriocytes, thus preventing direct symbiont contact with the host systemic immune system. I will address here recent advances in the understanding of the bacteriocyte immune and cellular regulations involved in endosymbiont maintenance and control. I will focus on the cereal weevils *Sitophilus* spp*.,* in which bacteriocytes form bacteriome organs that strikingly evolve in structure and number according to insect development and physiological needs. I will discuss how weevils track endosymbiont dynamics through at least two mechanisms: i- antimicrobial peptide synthesis that regulates endosymbiont cell cytokinesis and helps to maintain a homeostatic state within bacteriocytes, and ii- cellular processes such as apoptosis and autophagy, that adjust endosymbiont load to the host developmental requirements, hence ensuring a fine-tuned integration of symbiosis costs and benefits.

FEMS7-3296
Insect-microbe interactions

MOLECULAR MECHANISMS UNDERLYING DROSOPHILA SPIROPLASMA ENDOSYMBIOSIS

B. Lemaitre[1]
[1]*EPFL, Global Health Insitute, Lausanne, Switzerland*

Virtually every species of insect harbors facultative bacterial endosymbionts that are transmitted from females to their offspring, often in the egg cytoplasm. These symbionts play crucial roles in the biology of their hosts. Many manipulate host reproduction in order to spread within host populations. Others increase the fitness of their hosts under certain conditions, for example by increasing tolerance to heat or by protecting their hosts against natural enemies. We are dissecting the interaction between *Drosophila* and its native endosymbiont *Spiroplasma poulsonii*. *Spiroplasma* are members of the Mollicutes, a wall-less eubacterial group related to the Gram-positive lineage, which are very widespread and is likely to be present in over 5% of all insect species.

Our study has shown that *S. poulsonii* resides in large numbers in the hemolymph (the insect blood) of larvae and adults. Surprisingly *S. poulsonii* cells are neither detected nor affected by the *Drosophila* immune system, but their proliferation is constrained by the availability of hemolymph lipids. We hypothesize that this dependence on lipids couples the proliferation of *S. poulsonii* to the nutritional state of its host. We have also provided strong evidences that the ability of Spiroplasma to protect *Drosophila* against infestation by parasitoid wasps (a parasite of *Drosophila*) relies on a competition for host lipids. Recently, we have also shown that *Spiroplasma* uses the yolk uptake machinery to colonize the germ line, thus ensuring an efficient vertical transmission.

Spiroplasma is also a male killer, and it has been hypothesized that this reproductive manipulation is one of the driving forces that maintains this facultative endosymbiont in fly populations. In collaboration with the Fukatsu laboratory (Japan), we have shown that *Spiroplasma* targets the dosage compensation system of *Drosophila*, a machinery that is assembled only in males to regulate X chromosome expression. We are currently investigating in further details the mechanism of male killing. In parallel, our laboratory has sequenced the genome of several strains of *S. poulsonii* and developed new approaches to cultivate (for the first time) this bacterium *in vitro* and to transform it. These works pave the route to the genetic manipulation of this symbiont.

We believe that the fundamental knowledge generated on the *Drosophila-Spiroplasma* interaction will serve as a paradigm for other endosymbiont-insect interactions (ex. W*olbachia*) which are less amenable to genetic studies.

FEMS7-3282
Insect-microbe interactions

EXPERIMENTAL EPIDEMIOLOGY AND ANTIBIOTIC RESISTANCE TRANSMISSION

A. Moya[1,2,3]
[1]*Institute for Integrative Systems Biology, Evolutionary Systems Biology of Symbionts, Valencia, Spain*
[2]*CIBER, Epidemiology and Public Health, Madrid, Spain*
[3]*Foundation for the Promotion of Sanitary and Biomedical Research FISABIO, Genomicas and Health Area, Valencia, Spain*

Background

Antibiotic resistance is recognized as one of the major challenges in public health. The global spread of antibiotic resistance is the consequence of a constant flow of information across multi-hierarchical interactions, involving cellular (clones), subcellular (resistance genes located in plasmids, transposons, integrons) and supracellular (clonal complexes, genetic exchange communities, microbiotic ensembles) levels.

Objectives

In order to study such multi-level complexity, we propose to establish an experimental epidemiology model for the transmission of antibiotic resistance with the cockroach *Blatella germanica*

Methods

Here I report the results of a series of preliminary experiments with *B. germanica* populations that allow to conclude that this animal is an appropriate model for experimental epidemiology: a) the composition, transmission and acquisition of gut microbiota and endosymbionts*;* b) the effect of different diets on gut microbiota; c) the effect of antibiotics on host fitness; d) the evaluation of the presence of antibiotic resistance genes in natural- and lab-reared populations; and e) the preparation of plasmids harboring specific antibiotic resistance genes.

Conclusions

The basic idea is to have populations with higher and lower antibiotic exposure, simulating the hospital and the community, respectively, and with a certain migration rate of insects between populations. In parallel, we present a computational model based on P-membrane computing that will mimic the experimental system of antibiotic resistance transmission. The proposal serves as a proof-of-concept for the development of more complex population dynamics of antibiotic resistance transmission that are of interest in public health, that can evaluate procedures and design appropriate interventions in epidemiology.

FEMS7-0081
Microbial communities

MODELLING REGIME SHIFTS IN NUTRIENT-CYCLING MICROBIAL COMMUNITIES

T. Bush[1], I. Butler[2], A. Free[3], R.J. Allen[4]
[1]University of Edinburgh, School of Physics and Astronomy, Edinburgh, United Kingdom
[2]University of Edinburgh, School of Geosciences, Edinburgh, United Kingdom
[3]University of Edinburgh, School of Biological Sciences, Edinburgh, United Kingdom
[4], United Kingdom

Microbes play a crucial role in biogeochemical cycles on microscopic, macroscopic and planetary scales, by shuttling chemical elements between oxidized and reduced forms. Understanding and predicting how nutrient-cycling microbial ecosystems respond to environmental changes is therefore of great importance. In particular, we would like to know how and when “regime shifts”, or drastic changes in community function, happen. I will describe recent work on the development of mathematical models for simple sulphur-cycling microbial communities, in which we show that the response of a community to environmental change can be drastically different depending on the details of the microbial growth kinetics. In particular, saturation of the growth rate with respect to nutrient availability can lead to extreme sensitivity of community structure and function to environmental change.

FEMS7-0640
Microbial communities

DIVERSITY, STRUCTURE, AND NOVEL PHYSIOLOGIES IN MICROBIAL COMMUNITIES IN RAPID SAND FILTERS

B.F. Smets[1], A. Gülay[1], A. Palomo[1], S.J. Fowler[1], T. Sicheritz-Pontén[2]
[1]Technical University of Denmark, Dept of Environmental Engineering, Kgs Lyngby, Denmark
[2]Technical University of Denmark, Dept of Bio and Health Bioinformatics, Kgs Lyngby, Denmark

Rapid sand filtration (RSF) is frequently used to treat high-quality groundwater for drinking water production. Microbial density in RSFs is high and communities typically colonize the porous mineral matrix that coats the sand grains.
Microbes are assumed responsible for the removal of NH_4^+, Fe^{2+}, Mn^{2+}, CH_4, H_2S - the main constituents in groundwaters – although the details have remained obscure.
We have initiated a description of the diversity, abundance, and distribution of microbial communities across various RSFs, from which we have identified a core RSF microbiome, abundance of functional guilds, and spatial distribution patterns. Across all RSFs, *Nitrospira* abundance exceeded abundance of canonical Ammonium Oxidizing Bacteria (AOB).
Through metagenomic analysis, highly abundant composite population genomes belonging to the *Nitrospira* genus were recovered that harbor the metabolic capacity for complete ammonia oxidation (comammox).
DNA and RNA stable isotope probing (SIP) based on ^{13}C-labeled bicarbonate incorporation was subsequently performed on RSF samples under continuous loading and with nitrification inhibitors. SIP results provided the first insights into the *in situ* activity of comammox *Nitrospira* and novel nitrifiers in a complex microbiome.
Novel nitrifiers belonging to *α* and *β* proteobacteria were detected as central players in nitrification in RSFs, supported by evidence from SIP and metagenomics.
Here, we are obtaining a first comprehensive insight into the phylogeny and physiology of the microbes that drive the nitrogen cycle in RSFs.

FEMS7-1654
Microbiological macromolecular assemblies

CONTROL OF CELL MORPHOGENESIS IN ROD-SHAPED BACTERIA

C. Billaudeau[1], A. Chastanet[1], Z. Yao[1], C. Cornilleau[1], R. Carballido-Lopez[1]
[1]INRA, Micalis, Paris, France

Control of cell morphogenesis in rod-shaped bacteria

How cells control their shape and size is a long-standing question in cell biology. Many rod-shaped bacteria elongate their sidewalls by the action of cell wall synthesizing machineries that are associated to actin-like MreB dynamic cortical patches. However, little is known about how elongation is regulated to enable varied growth rates and sizes. We have used total internal reflection fluorescence microscopy (TIRFM) combined with automated single particle tracking (SPT) analysis to visualize the dynamics and properties of MreB isoforms, as a proxy for cell wall synthesis, in cells of the model Gram-positive bacterium *Bacillus subtilis* and of the model Gram-negative *Escherichia coli* growing under different nutrient conditions and upon nutrient shift. We show that although both organisms present a similar mechanism of cell elongation they use orthogonal strategies to adapt to growth regime variations. We have also shed light on the current controversy of whether (and why) MreB proteins assemble into diffraction-limited structures or elongated filaments, and used super-resolution microscopy (TIRF-SIM) to elucidate the nanostructure of MreB assemblies.

FEMS7-2445
Microbiological macromolecular assemblies

STRUCTURE AND FUNCTION OF CONTRACTILE INJECTION SYSTEMS

P. Leiman[1]
[1]*University of Texas Medical Branch, Department of Biochemistry and Molecular Biology, Galveston, USA*

Backgrounds

The process of protein and DNA translocation across lipid membranes is central to the function of any organism. The Type VI Secretion System (T6SS) translocates a range of substrates that vary in their size, properties, and secretion selection mechanisms into the external milieu and sometimes directly into the cytoplasm of neighboring cells. T6SS is orthologous to a contractile tail of a bacteriophage, which is used by the phage for translocation of proteins and DNA in the opposite direction — from the capsid located outside of the cell into the cell's cytoplasm. T6SS and phage tails consist of a sheath enveloping a tube with a spike-shaped protein at its tip. T6SS-secreted substrates are associated with the spike or loaded into the tube. The sheath in this assembly is a stretched-out spring that can contract and drive the tube and the spike through the cell envelope, and this constitutes the translocation event. The sheath-tube structure is locked in a high energy metastable state by the baseplate, which is also responsible for triggering the contraction of the sheath.

Objectives

To describe the mechanism of the structural transformation of the sheath in quantitative terms and relate it to T6SS and phage tail function.

Methods

Molecular modeling of the atomic structure that was obtained with the help of cryo-electron microscopy.

Conclusions

The contraction is accomplished by a massive structural rearrangement of the sheath in which its mesh-like topology is preserved and the subunits move as rigid bodies.

FEMS7-0072
Microbiological macromolecular assemblies

STRUCTURAL AND MOLECULAR BIOLOGY OF BACTERIAL SECRETION SYSTEMS
G. Waksman[1]
[1], *United Kingdom*

Structural and Molecular Biology of Type IV Secretion Systems

Gabriel Waksman[1]

[1] Institute of Structural and Molecular Biology at UCL and Birkbeck, Malet Street, London, WC1E 7HX, UK

Type IV secretion (T4S) systems are molecular machines used for the transport of macromolecules across the bacterial cell envelope. T4S systems are highly versatile. Conjugative T4S systems translocate DNA from a donor to a recipient bacterium and contribute to bacterial genome plasticity, spread of antibiotic resistance or other virulence trait among bacterial pathogens. In some bacteria such as *Helicobacter pylori* (Cag PI), *Brucella suis* (VirB/D), or *Legionella pneumophila* (Dot, Icm), T4S systems are directly involved in pathogenicity as they mediate the secretion of virulence factors (DNA or toxins) into host cells. The archetypal T4S system, the VirB/D system, was defined in *Agrobacterium tumefaciens* where it is naturally responsible for the delivery of the T-DNA to the plant host-cell. Conjugative T4SS as well as the *A. tumefaciens* VirB/D system comprise 12 proteins (VirB1 to 11 and VirD4). Recently, structures of large complexes formed by several of these proteins and their substrates have become available for conjugative T4S systems shedding unprecedented light on T4S system secretion mechanism.

Key references

1- Chandran, R. Fronzes, S. Duquerroy, N. Cronin, J. Navaza, and G. Waksman (2009). Nature. 462:1011-1015.

2- R. Fronzes, E. Schaefer, H. Saibil, E. Orlova and G. Waksman (2009). Science. 323:266-268.

3- H.H. Low, F. Gubellini, A. Rivera-Calzada, N. Braun, S. Connery, A. Dugeancourt, F. Lu, A. Redzej, R. Fronzes, E.V. Orlova, and G. Waksman (2014). Nature. 508: 550–553.

4- T. Costa, A., Ilangovan, M. Ukleja, A. Redzej, J. Santini, T. Smith, E. Egelman and G. Waksman (2016). Structure of the bacterial sex F pilus reveals an assembly of a stoichiometric protein-phospholipid complex. Cell. 166, 1436–1444.

FEMS7-0069
Microbiota-host interactions

ROLE OF GUT MICROBIOTA IN RESPONSE TO STRESS

T. Dinan[1]
[1]*University College Cork, Department of Psychiatry and APC Microbiome Institute, Cork, Ireland*

Role of gut microbiota in response to stress

Ted Dinan MD, PhD, Department of Psychiatry and APC Microbiome Institute, University College, Cork

Evidence is accumulating to suggest that gut microbes may be involved in neural development and function, both peripherally in the enteric nervous system and centrally in the brain. While evidence is still limited in psychiatric illnesses, there are rapidly coalescing clusters of evidence which point to the possibility that variations in the composition of gut microbes may be associated with changes in the normal functioning of the nervous system. Studies in germ-free animals indicate aberrant development of the brain monoaminergic system together with memory deficits and autistic patterns of behaviour. These deficits can be partially normalised if there is early gut colonisation.

There are marked differences in the gut microbiota between patients with major depression and healthy controls. Following a cocktail of antibiotics we conducted a faecal microbiota transplant in rats with faeces from depressed patients or healthy controls. Those rats receiving a transplant from depressed patients developed a depressive phenotype with alteration in corticosterone release and tryptophan metabolism.

That bacteria might have a positive mental health benefit is now becoming clear. Such bacteria may influence the capacity to deal with stress, reducing anxiety, perhaps positively impacting on mood and are now called psychobiotics. Whether, they are capable of acting like and in some circumstances replacing antidepressants remains to be seen. The mechanisms of psychobiotic action are gradually being unravelled. It has been show that *Lacobacillus rhamnosus* has potent anti-anxiety effects in animals and does so by producing major changes in the expression of GABA receptors in the brain. The changes in these receptors are mediated by the vagus nerve which connects the brain and gut. When this nerve is severed no effect on anxiety or on GABA receptors is seen following psychobiotic treatment.

Communication between the brain and gut is bidirectional and complex. Increased understanding of this axis and the role of the gut microbiota may aid the development of therapies not just for functional bowel disorders but for mood disorders also.

FEMS7-0859
Microbiota-host interactions

THE ROLE OF THE EARLY LIFE MICROBIOTA ON HOST HEALTH

L. Hall[1]
[1]*Institute of Food Research, Gut Health & Food Safety, Norwich, United Kingdom*

A 'healthy' intestinal epithelial barrier is essential for host wellbeing and is maintained through a tight monolayer of intestinal epithelial cells (IECs) and balanced interactions with the resident microbiota. Notably a breakdown of this interface has been associated with intestinal inflammatory pathologies including Inflammatory Bowel Disease (IBD), and may be linked to alterations in the microbiota, including a reduction in *Bifidobacterium*. Here I will discuss our data which suggests that *Bifidobacterium* can positively influence epithelium responses *in vivo* during both health and disease via specific modulation of the IEC transcriptome and regulatory networks, including downregulation of cell death pathways. Using KO mouse models and mutant bacterial strains we have uncovered key interactions between bifidobacterial (i.e. exopolysaccharide) and host (i.e. MyD88) molecules, which serve to reduce pathological epithelial cell injury. These data highlight the importance of maintaining healthy homeostatic cross-talk with specific microbiota members and open up mechanistic pathways that could be targeted for future therapies.

FEMS7-0097
Microbiota-host interactions

DISSECTING CAUSALITY OF FECAL MICROBIOTA IN HUMAN METABOLISM AND OBESITAS

M. Nieuwdorp[1]
[1], *Amsterdam, The Netherlands*

Alterations in (small) intestinal microbiota are associated with obesity and insulin resistance, with the latter usually characterized by low grade endotoxemia. Recent intervention stuies showed that specific bacterial species in fecal samples are altered upon treatment with metformin in type 2 diabetes mellitus patients (Forslund, Nature 2015). We previously showed that fecal transplantation (infusing intestinal microbiota from lean donors) in male recipients with metabolic syndrome has beneficial effects on the recipients' microbiota composition and glucose metabolism via lowering plasma endotoxin levels (Vrieze, Gastroenterology 2012) and followup studies suggest that this beneficial effect can be divided in responders (70%) and non responders (30%) based on butyrate producing microbiota engraftment (Kootte, manuscript in preparation). Intruigingly, oral treatment with sodiumbutyrate for 4 weeks only induced beneficial effects on insulin sensitivity only in healthy subjects whereas no effects are seen in metabolic syndrome subjects (Bouter, manuscript in preparation). Combined our data suggest that specific intestinal bacterial strains might be developed as therapeutic for cardiometabolic diseases such as insulin resistance, NASH and obesity and these probiotics will most likely be superior to orally providing beneficial metabolites. Moreover, it is expected that the driving mechanisms might be driven by immunological, genetic and microbiota based factors.

FEMS7-1160
Mosquito-borne viruses

WEST NILE VIRUS IN EUROPE

B. Luisa[1]
[1]*University of Padova, Department of Molecular Medicine, Padova, Italy*

West Nile virus (WNV) is a mosquito-borne flavivirus that has recently re-emerged in Europe. First isolated in Uganda in 1937, the virus was responsible for epidemic outbreaks in Africa and in the Middle East and of sporadic infections in Europe until 1996, when a large outbreak of neuroinvasive disease occurred in Romania. Since then, almost all Eastern, Central, and Southern European Countries reported human outbreaks and sporadic cases, especially during the recent years. Phylogenetic analyses of WNV lineage 1 and lineage 2 strains that circulate in Europe showed that, notwithstanding a high genetic diversity, these strains are derived from a limited number of independent introductions, most likely from Africa through migrating birds, followed by local spread and evolution. Following the re-emergence of WNV, European countries have intensified surveillance activities and control programs. However, the dynamics of virus re-emergence and spread and the mechanisms involved in its establishment into new areas are still poorly understood. Improvements have been also made in the diagnosis of WNV infection with the development of new laboratory tests, but the diagnosis remains challenging and many cases are not recognized. Important aspects of WNV pathogenesis have been clarified in experimental models and this information is crucial for the development of vaccines and antiviral drugs. However, the mechanisms involved in human disease are still largely unknown and require further research. The novel findings on the ecology, molecular epidemiology, pathogenesis, clinical features, and diagnosis of WNV infection in humans will be discussed, with special focus on Europe.

FEMS7-0355
Mosquito-borne viruses

THE EVOLUTION OF WEST NILE VIRUS IN THE USA AND THE ROLE OF VECTOR-VIRUS INTERACTIONS IN PATTERN OF TRANSMISSION

A. Ciota[1], S. Bialosuknia[2], L. Kramer[2]
[1], USA
[2]Wadsworth Center- New York State Department of Health,
Division of Infectious Diseaes- Arbovirus Laboratory, Slingerlands- NY, USA

West Nile virus (WNV; *Flaviviridae, flavivirus*) has been endemic in New York State (NYS) since its 1999 introduction, yet prevalence in Culex mosquitoes varies substantially over small spatial and temporal scales. It is unclear if viral genetics plays a role in this variability, as genetic and phenotypic characterization on local scales has generally been lacking. In addition, mutant swarms of circulating strains have not been fully characterized despite the documented role of minority variants in viral fitness and virulence. In an effort to characterize WNV variability within epidemiologically relevant scales, we performed phylogenetic analyses on WNV isolates from the U.S. obtained from1999-2015 and focused exclusively on New York State isolates. In addition, we performed full-genome, deep-sequencing and genetic analyses on 15 WNV isolated in 2012 from *Cx. pipiens* from an endemic focus in Suffolk County, NY. Our results indicate continued evolution and seasonal maintenance, yet also widespread mixing and high levels of genetic diversity within geographic foci and individual seasons throughout the U.S. Well supported local clusters with shared amino acid differences were identified and suggest local evolutionary pressures and the potential for phenotypic variability. Intra-host diversity of focal isolates was also high, with polymorphism at levels >1.0% identified in approximately 10% of the WNV genome. Although most minority mutations were not shared, mutational hotspots shared among local isolates were identified, particularly in C, NS1 and NS2A genes. The most polymorphic region, positions 3198-3388 of the NS1 gene, was comprised predominately of non-synonymous mutations, suggesting a selective advantage for amino acid diversity in this region. The data provide insight into the movement and local evolutionary pressures of WNV in mosquitoes, and provide a baseline to study the role of genetics in regional variability of WNV prevalence and disease.

FEMS7-1419
Mosquito-borne viruses

DENGUE-MOSQUITO INTERACTIONS
L. Lambrechts[1]
[1]*Institut Pasteur, Genomes and Genetics, Paris, France*

Within-host evolution of dengue viruses in their mosquito vector

Louis Lambrechts
Department of Genomes & Genetics, Institut Pasteur-CNRS, Paris, France

Abstract

Like other pathogens with high mutation rate and rapid replication, dengue viruses evolve during the course of an infection. We used deep sequencing to monitor the evolutionary dynamics of dengue virus populations during infection of several genetic backgrounds of their main mosquito vector *Aedes aegypti*. Our results showed that initial infection of the mosquito's midgut was randomly founded by only a few tens of individual virus genomes. The overall level of viral genetic diversity generated during infection was predominantly under purifying selection but differed significantly between mosquito genotypes. Thus, in addition to random evolutionary forces and the purging of deleterious mutations that shape dengue virus genetic diversity during vector infection, our results also pointed to a novel role for vector genetic factors in the genetic breadth of virus populations. Together, our findings illustrate how identifying the evolutionary forces acting on dengue virus populations within the mosquito can shed light on vector-virus interactions and between-host viral evolution.

FEMS7-2595
Protein folding and protein secretion pathways

REGULATION AND FUNCTION OF TYPE III EFFECTORS FROM XANTHOMONAS

U. Bonas[1]
[1]*Martin-Luther-Universität, Institut für Biologie/Genetik, Halle, Germany*

Pathogenicity of most Gram-negative plant-pathogenic bacteria depends on the type III secretion (T3S) system which translocates effector proteins (T3Es) into the eukaryotic cell cytosol. We study the interaction between *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) and its host plants pepper and tomato. The bacteria enter the plant tissue via natural openings or wounds and settle down in the intercellular spaces; they do not invade the plant cells. Plant cell contact and special media induce the expression of the T3S system components and most T3Es. In susceptible plants, T3Es interfere with host cell processes to the benefit of the pathogen and allow its proliferation. In resistant plants, single resistance genes mediate recognition of individual T3Es often resulting in the induction of a hypersensitive response (HR), a rapid and localized programmed cell death restricting bacterial growth. *Xcv* injects more than 30 different T3Es into the plant cell, termed Avr (avirulence protein) if they were identified many years ago, or Xop (Xanthomonas outer protein). Among the T3Es from *Xcv* are plant immunity suppressors, cell death inducers, a ubiquitin ligase, the transcription factor AvrBs3 (a "TALE") and proteins of unknown function. Selected T3Es will be discussed.

FEMS7-2924
Protein folding and protein secretion pathways

ASSEMBLING COMPLEXES BETWEEN B-BARRELS AND LIPOPROTEIN

S.H. Cho[1], J.F. Collet[2]
[1]de Duve, Biochemistry, Brussels, Belgium
[2]Université catholique de Louvain, de Duve Institute, Brussels, Belgium

Backgrounds

Bacterial lipoproteins are a very diverse group of proteins characterized by the presence of an N-terminal lipid moiety that serves as a membrane anchor. Lipoproteins have a wide variety of crucial functions, ranging from envelope biogenesis to stress response. In Gram-negative bacteria, lipoproteins can be targeted to various destinations in the cell, including the periplasmic side of the cytoplasmic or outer membrane, the cell surface or the external milieu.

Objectives

One of the objectives of our lab is to understand how lipoproteins can reach the cell surface of Escherichia coli and other Gram-negative bacteria.

Methods

By focusing on the lipoprotein stress sensor RcsF, we dissected the molecular mechanism allowing this protein to become surface-exposed.

Conclusions

FEMS7-1645
Protein folding and protein secretion pathways

THE TYPE VI SECRETION SYSTEM: A BACTERIAL KILLING MACHINE

A. Filloux[1]
[1]*Imperial College London, Department of Life Sciences, London, United Kingdom*

The type VI secretion system (T6SS) is a weapon of bacterial warfare and host cell subversion. The Gram-negative pathogen *Pseudomonas aeruginosa* has three T6SSs involved in colonization, competition and full virulence. The H1-T6SS is a molecular gun firing seven toxins, Tse1-7, challenging survival of other bacteria and helping *P. aeruginosa* to prevail in specific niches.The characterization of the T6SS structure largely benefited from the knowledge acquired from the phage tail molecular details. Indeed the T6SS has been proposed to be a supra-molecular bacterial complex that resembles phage tails. The T6SS nanomachine thus include a tail tube or Hcp, a puncturing device or VgrG and a contractile sheath made of TssBC. Contraction of the sheath propels the tail tube and the puncturing device on which is associated the various toxins into the prey cells. The baseplate-like component of the phage tail is yet to be characterized in the T6SS. Striking similarities are emerging which make possible the elaboration of a model and further understanding of the evolutionary pathways which have distinguished two molecular devices similar in structure but distinct in function.

FEMS7-0118
Protein folding and protein secretion pathways

SPATIOTEMPORAL ORGANISATION IN THE BACTERIAL OUTER MEMBRANE AND ITS ROLE IN COLICIN IMPORT

C. Kleanthous[1]
[1], *United Kingdom*

Bacteriocins are species-specific protein antibiotics deployed by Gram-negative bacteria to kill their neighbours during competition for resources. They are implicated in the stable co-existence of bacteria within microbiomes, are exploited by pathogens and commensals alike and are effective therapeutics in animal models of infection. Protein bacteriocins (~40-60 kDa) kill susceptible organisms by binding to a specific receptor and translocating a cytotoxic domain (an enzyme or ionophore) across the cell envelope through a series of protein-protein interactions. We have been exploiting colicins, bacteriocins specific for *Escherichia coli*, as probes of the structure and architecture of the cell envelope and in particular the outer membrane. Using non-translocating colicins as high affinity labels of outer membrane proteins (OMPs) in live bacterial cells, we have shown recently that OMPs are both spatially and temporally organized and that this organization lies at the heart of how OMPs are turned over, which answers a longstanding question in the field. Spatiotemporal OMP organization is a new concept with unknown biological ramifications. My talk will focus on recent studies where we demonstrate that the impact of spatiotemporal OMP organization can be felt across the cell envelope and that bacteriocins likely exploit spatiotemporal OMP organization in order to translocate into Gram-negative bacteria.

FEMS7-1038
Resistome

THE SOIL RESISTOME

P. Simonet[1], *J. Nesme*[1], *E. Topp*[2]
[1]*Ecole Centrale de Lyon, Ampère, Ecully, France*
[2]*Agriculture and Agri-Food Canada, Department of Biology, London, Canada*

Does application of antibiotic molecules to farm soil significantly increase resistance genes abundance?

In a context where pathogens acquire resistance to more and more antibiotic treatments in hospital settings a number of questions are raised regarding mobilization, acquisition and dissemination of antibiotic-resistance traits between and within bacterial populations. Since antibiotic resistance genes (ARG) are common features of many saprophytic soil bacteria a significant ARG reservoir is readily accessible to previously sensitive pathogens by means of horizontal gene transfer (HGT). It is known since decades that environmental bacteria and pathogens share resistance genes hence the importance to clearly establish transmission routes of such traits in order to prevent their dissemination.

Crop fertilization using manure from animals previously treated with antibiotics is a major anthropogenic entry point of these pharmaceuticals in soils. A number of risk-assessment studies focused on the effect of manure fertilization on soil bacterial community and showed that such practice can indeed increase ARG abundance. Such studies are however most often depicting short- to mid-term impact and cannot distinguish what is leading increase in resistance genes abundance. Our objective was therefore to assess if long-term (1999-present) impact of repeated antibiotic molecules input would have a similar impact on soil bacterial communities, in absence of manure. Can similar concentrations of antibiotic select for resistance traits and increase their dissemination potential in absence of organic matter and intrinsic bacterial community added to soil *via* manure?

To test this hypothesis we analyzed environmental DNA extracted from soil samples under crop rotation and treated yearly with veterinary antibiotics (tylosin, chlortetracycline and sulfonamides) for 15 years (1999-present). Antibiotic molecule concentration added to soil each year represented similar amount to what is conventionally found in antibiotic-treated animal manure. Contaminated soil plots examined in this analysis represents a 12 years time frame (2001 - 2012) of this long-term experimental setup. No correlation between increased antibiotic concentration added to soil and antibiotic-resistance gene abundance could be established with our results and the soil bacterial community is not significantly affected by such treatment. Investigation of ARG and mobile genetic element promoting gene transfer between bacterial lineages (*i.e.*: from environmental bacteria to pathogens) has also been analyzed. It shows that co-abundance is frequent in sequence datasets obtained from soil samples. However, new sequence analysis methods are needed to determine if co-abundance is indeed a sign of co-occurrence on the same molecule, explaining increased transfer potential and successful ARG dissemination.

FEMS7-1181
Resistome

NOVEL TECHNOLOGIES TO STUDY THE RESISTOME

W. van Schaik[1]
[1]*University of Birmingham, Institute of Microbiology and Infection, Birmingham, United Kingdom*

The human gut microbiome is a complex ecosystem, containing hundreds of bacterial species. While many of these species have a symbiotic or commensal relation with their host, the human gut also serves as a reservoir for opportunistic pathogens and antibiotic resistance genes ('the resistome'). Culture-independent analyses of metagenomic DNA have revealed that antibiotic resistance genes are common in the gut microbiota of both healthy individuals and hospitalised patients. The resistome can be characterized by a variety of culture-independent analyses, including high-throughput quantitative PCR, metagenomic shotgun sequencing and functional metagenomics (i.e. the generation of large clone libraries that can be screened for antibiotic resistant clones).
I will present studies from my group in which these different techniques were used to characterize the resistome of hospitalised patients that received intensive antibiotic therapy. These data show that the resistome is highly dynamic under antibiotic therapy and that commensal bacteria (e.g. Bacteroidetes and Clostridium) are major reservoirs of antibiotic resistance genes in the human gut. Finally, I will discuss the pros and cons of different approaches to characterize the resistome and present to what extent rapid resistome characterisation can be used to guide targeted antibiotic therapy and/or infection control measures.

FEMS7-3290
Resistome

METAGENOMICS AND CULTUROMICS

J. Holden[1], S. Rangama[1], I. Lidbury[1], V. Clark[1], S. Djordjevic[2], P. Hawkey[3], E. Wellington[1]
[1]University of Warwick, School of Life Sciences, Coventry, United Kingdom
[2]University of Technology Sydney, The ithree institute, Sydney, Australia
[3]Heartland Hospital and University of Birmingham, Institute of Microbiology and Infection, Birmingham, United Kingdom

How are antimicrobial resistance genes disseminated into the environment and help their hosts survive?

Infections caused by antimicrobial resistant bacterial pathogens are global and represent an increasingly important public health problem. A critical component in controlling dissemination is the identification of pathogen reservoirs. We have identified the environment as a further potential component of the pathogens' complex transmission cycle. Once outside the enriched mammalian host (or culture media), cells can be in an altered physiological state, as pathogens can enter a resilient but quiescent state in order to survive the biotic and abiotic stresses of the environment. Culture independent methods are required to quantify these cells, and culture dependant methods have been relied on to establish if they are viable and potentially capable of causing infection. However many pathogens can survive and grow in the environment and genetically interact with indigenous environmental bacteria or protozoans. In addition with the increase in surgical procedures and improvements in medical care certain bacteria regarded as 'environmental' have caused infections and been exposed to antibiotics and disinfectants. Waste water treatment plants (WWTPs) and manure treatments can spread antimicrobial resistant (AMR) gut bacteria into the environment usually in mixtures with amoebae, other protozoans, and comprise both pathogenic and commensal bacteria[1-3]. The fate of these bacteria is uncertain and their turnover difficult to determine due to continued inputs and depletion via natural processes. What is clear is that many pathogenic *E. coli* are capable of surviving long periods of time in hostile environments and carry multi-drug resistance plasmids some with pathogenicity determinants which are readily mobilised to other host backgrounds if conditions are appropriate.

The genomes of a range of *E. coli* strains isolated from downstream of WWTP effluent outflows provided evidence of extensive conservation of traits and prevalence of IncFIA, IncFIB replicon types and these were further investigated for plasmid and gene diversity, fitness and gene expression. Results revealed differences in fitness putatively associated with plasmid presence and variations in the exoproteome and resistance gene expression related to gene location and growth conditions. We hypothesize that plasmids are integral to strain fitness and survival[4].

1. Amos GC, Gozzard E, Carter CE, et al. Validated predictive modelling of the environmental resistome. *ISME J* 2015; **9**(6): 1467-76.

2. Amos GC, Hawkey PM, Gaze WH, Wellington EM. Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. *J Antimicrob Chemother* 2014; **69**(7): 1785-91.

3. Amos GC, Zhang L, Hawkey PM, Gaze WH, Wellington EM. Functional metagenomic analysis reveals rivers are a reservoir for diverse antibiotic resistance genes. *Vet Microbiol* 2014; **171**(3-4): 441-7.

4. Yano H, Wegrzyn K, Loftie-Eaton W, et al. Evolved plasmid-host interactions reduce plasmid interference cost. *Mol Microbiol* 2016.

FEMS7-0053
Synthetic microbiology

SYNTHETIC APPROACHES OF TRANSLATIONAL CONTROL

K. Jung[1], *W. Volkwein*[1], *J. Lassak*[1]
[1]*LMU Munich, Biology, Martinsried, Germany*

Aims:

Our studies aim to create a generic EF-P variant, which is standardized and constitutively active independent of species-specific posttranslational modifications. Furthermore, we aim to develop a new toolbox for the translational control of protein levels.

Methods:

The amber suppression system is used to incorporate unnatural amino acids. All synthetic protein variants are tested for functionality *in vivo* using reporter strains.

Results:

Translation of proteins with a stretch of consecutive prolines causes ribosome stalling. To overcome this stop, bacteria depend on a specific translation elongation factor P (EF-P), being orthologous and functional identical to eukaryotic/archaeal elongation factor e/aIF-5A (1-3). EF-P binds to the ribosome between the peptidyl-tRNA binding site (P-site) and the tRNA exiting site (E-site) and stimulates peptide bond formation. In their active form both EF-P and e/aIF-5A are post-translationally modified at a positively charged amino acid, which protrudes towards the peptidyl-transferase center. While archaeal and eukaryotic IF-5A depend on hypusination of a conserved lysine, the EF-P modification strategies in bacteria vary. In *Escherichia coli* and *Salmonella enterica* a lysine of EF-P is extended by β-lysinylation and subsequently hydroxylated, whereas in *Pseudomonas aeruginosa* and *Shewanella oneidensis* an arginine in the equivalent position is rhamnosylated (4). In addition to structural constrains of polyproline stretches, some EF-P dependent proteins require this motif to fine-tune the protein output. We replaced the conserved lysine with various unnatural amino acids, and screened for active EF-P variants *in vivo* using reporter strains.

In parallel, a new toolbox for the translational control of proteins was generated. This toolbox is based on the combination of amber suppression and stalling motifs and allows tight translational control of protein levels in various bacterial species.

Conclusion:

Translational control allows fine-tuning of the protein output.

References:
(1) Ude S, Lassak J, Starosta AL, Kraxenberger T, Wilson DN Jung K (2013) Translation elongation factor EF-P alleviates ribosome stalling at polyproline stretches.

(1) Ude S, Lassak J, Starosta AL, Kraxenberger T, Wilson DN Jung K (2013) Translation elongation factor EF-P alleviates ribosome stalling at polyproline stretches. *Science* **339**, 82-85.

(2) Doerfel LK, Wohlgemuth I, Kothe C, Peske F, Urlaub H, Rodnina MV (2013) EF-P is essential for rapid synthesis of proteins containing consecutive proline residues. *Science* **339**, 85-88.

(3) Gutierrez E, Shin B, Woolstenhulme C, Kim J, Saini P, Buskirk A, Dever T (2013) eIF5A promotes translation of polyproline motifs. *Mol. Cell* **51**, 1-11.

(4) Lassak J, Keilhauer EC, Fürst M, Wuichet K, Gödeke J, Starosta AL, Chen J, Søgaard-Andersen L, Rohr J, Wilson DN, Häussler S, Mann M, Jung K (2015) Arginine-rhamnosylation as new strategy for post-translational modification of translation elongation factor P, *Nat. Chem. Biol.* **11**, 266-270.

FEMS7-0290
Synthetic microbiology

FINE AND SPECIALITY CHEMICAL PRODUCTION USING SYNTHETIC BIOLOGY

E. Takano[1]
[1]*University of Manchester, Manchester Institute of Biotechnology, Manchester, United Kingdom*

We aim to design and construct organisms with new functionalities of unprecedented scope, by exploiting synthetic biology for metabolic engineering; e.g. by harnessing our ability to readily sequence complete genomes and to rewrite/re-design pathways on a large scale.

We explore these possibilities in the context of high-value chemical production, utilising the Design/Build/Test/Learn cycle at the Parts, Devices and Systems level. Many microorganisms already have the machinery to produce diverse bioactive molecules that can be used in health, agriculture and food (Cimermancic et al., 2014). As a first step towards re-engineering these high-value chemical biosynthesis pathways for enhanced productivity and diversity, we aim to understand the interchangeability of biosynthetic parts (Diez et al., 2015) and created a minimal information database for natural products with the support from the natural products community (Medema et al., 2015). We have designed and assembled pathways using the identified parts (Leferink et al., 2016) and will engineer orthogonal transcription mechanisms (based on signalling molecule circuits (Biarnes-Carrera et al., 2015) and bacterial microcompartments (Chessher et al., 2015). In addition, we are expanding our collection of computational tools for the detection and analysis of secondary metabolite biosynthesis gene clusters, to enrich our library of parts and building blocks for pathway engineering (Weber et al., 2015). We also use computational modelling (constraint-based descriptions of bacterial metabolism) to identify suitable overproduction hosts and pinpoint biosynthetic bottlenecks to target for further cellular engineering in a synthetic biology strategy (Breitling et al., 2013). And finally, we combine this analysis with high-resolution mass spectrometry analysis, which we also employ for the debugging of the engineered systems (Jankevics et al., 2012).

We have these tools in the Design/Build/Test/Learn cycle of the recently established BBSRC/EPSRC-funded Manchester Synthetic Biology Research Centre, SYNBIOCHEM, where they provide a platform for the high-throughput engineering of fine and speciality chemicals production in microbial systems.

References:

Biarnes-Carrera M, Breitling R, **Takano E.** Butyrolactone signalling circuits for synthetic biology. ***Curr. Opin. Chem. Biol.*** (2015) 28:91-98.

Breitling R, Achcar F, **Takano E** Modeling challenges in the synthetic biology of secondary metabolism. ***ACS Synth. Biol.*** (2013) 19:373-378.

Chessher A, Breitling R, **Takano E**. Bacterial microcompartments: biomaterials for synthetic biology-based compartmentalization strategies. ***ACS Biomater. Sci. Eng.*** (2015) 1:463-616.

Cimermancic P, Medema MH, Claesen J, Kurita K, Wieland Brown LC, Mavrommatis K, Pati A, Godfrey PA, Koehrsen M, Clardy J, Birren BW, **Takano** E, Sali A, Linington RG, Fischbach MA. Insights into secondary metabolism from a global analysis of bacterial biosynthetic gene clusters. ***Cell*** (2014) 158:412-21. ***highlighted in Nature Chemical Biology, 10 798-800 (2014)***

Diez V, Loznik M, …, **Takano E**. Functional exchangeability of oxidase and dehydrogenase reactions in the biosynthesis of hydroxyphenylglycine, a non-ribosomal peptide building block. ***ACS Synth Biol*** (2015) 4:796–807

Jankevics A, Merlo ME, de Vries M, Vonk RJ, **Takano E**, and Breitling R. Separating the wheat from the chaff: a prioritisation pipeline for the analysis of metabolomics datasets. ***Metabolomics*** (2012)8:29-36.

Leferink NGH, Jervis AJ, Zebec Z, Toogood HS, Hay S, **Takano E**, Scrutton NS. A 'plug and play' platform for the diverse monoterpene hydrocarbon scaffolds in *Escherichia coli*. ***ChemistrySELECT*** (2016) DOI10.1002/ slct.20600563

Medema MH, Kottmann R, Yilmaz P, Cummings M, [146 co-authors in alphabetical order], Breitling R, **Takano E**, Glöckner FO: The Minimum Information about a Biosynthetic Gene cluster (MIBiG) specification. ***Nature Chem. Biol***. (2015) 11: 625-631.

Weber T, Blin K, …, **Takano E**, Medema MH. antiSMASH 3.0-a comprehensive resource for the genome mining of biosynthetic gene clusters. ***Nucl. Acids Res.*** (2015) PMID:25948579

FEMS7-3324
Systems microbiology

METAGENOMICS AND SYSTEMS MICROBIOLOGY

P. Bork[1]
[1]*EMBL, SCB, Heidlebeg, Germany*

Microbiomics at different scales: From the human gut to the ocean

The human gut microbiome can now be readily studied using metagenomics (Qin et al., Nature 2010) and harbours more than 1000 species that are associated with important functions, but also with more than 30 common human diseases. I touch upon a few community properties such as species interactions or resilience. Perturbations such antibiotics intake, but also that of other drugs can have a lasting impact (e.g. Forslund et al., Nature 2015) as has faecal microbiota transplantation (Li et al., Science 2016). Using single nucleotide variation (Schloissnig et al. 2013) we can monitor at high resolution specific commensal or pathogenic strains in stool samples, for example in response to diet, but also learn, how the gut microbes colonise us, at birth and after. As most of our gut microbes are coming from the environment, it is crucial to study biodiversity and interactions of microbes at planetary scale. The feasibility of such a global approach is illustrated by the TARA oceans project surveying the microbial diversity of this vast ecosystem by studying plankton from 35000 samples collected from all major ocean regions (Bork et al., Science 2015 and refs therein).

FEMS7-2607
Systems microbiology

EVOLUTION OF TRANSCRIPTIONAL REGULATION IN BACTERIA

M.S. Gelfand[1]
[1]*A.A.Kharkevich Institute for Informatin Transmission Problems, RTC Bioinformatics, Moscow, Russia*

Comparative genomic techniques applied to dozens of sequenced bacterial genomes allow for highly reliable computational identification of transcription-factor (TF) binding sites and RNA regulatory elements. This, in turn, creates an opportunity for the analysis of evolution of regulatory networks and co-evolution of TFs and TF-binding DNA motifs. The regulatory interactions turn out to be extremely labile, and while the general principles of regulatory evolution are yet to be discovered, it is clear that it may involve large-scale network restructuring involving loss, gain, and duplication of TFs; breakdown, contraction, and expansion of regulons; conversion of local regulators into global ones etc. Similarly, integration of TF phylogenetic trees and data on their DNA motifs yields identification of co-evolving positions that frequently turn out to form contacts in 3D structure.

FEMS7-0350
Systems microbiology

THEORY OF MICROBIAL GENOME EVOLUTION

E.V. Koonin[1]
[1], *USA*

Bacteria and archaea have small genomes tightly packed with protein-coding genes. This compactness is commonly perceived as evidence of adaptive genome streamlining caused by strong purifying selection in large microbial populations. However, by comparing predictions of population-genetic models to comparative genomic data, we show that new genes acquired by microbial genomes, on average, are adaptive. Evolution of bacterial and archaeal genomes is highly dynamic and is dominated by horizontal gene transfer and gene loss. Many microbes have open pangenomes, where each newly sequenced genome contains more than 10% 'ORFans', genes without detectable homologues in other species. A quantitative analysis of microbial genome evolution using a simple, steady-state evolutionary model reveals two sharply distinct classes of microbial genes, one of which (ORFans) is characterized by effectively instantaneous gene replacement, whereas the other consists of genes with finite, distributed replacement rates. These findings imply a conservative estimate of the prokaryotic genomic universe size, which appears to contain at least a billion distinct genes.

References:

Sela I, Wolf YI, Koonin EV. Theory of prokaryotic genome evolution. Proc Natl Acad Sci U S A. 2016 Oct 11;113(41):11399-11407

Wolf YI, Makarova KS, Lobkovsky AE, Koonin EV. Two fundamentally different classes of microbial genes. Nat Microbiol. 2016 Nov 7;2:16208

FEMS7-2923
Systems microbiology

ENZYME AND METABOLITE CONCENTRATIONS ARE TIGHTLY BALANCED TO OPTIMALLY UTILIZE THE CYTOSOL'S SOLVENT CAPACITY

H. Dourado[1], V. Maurino[2], M. Lercher[1]
[1]Heinrich Heine University, Computer Science and Biology, Düsseldorf, Germany
[2]Heinrich Heine University, Biology, Düsseldorf, Germany

Title: *In vivo* Enzyme and Substrate Concentrations are Balanced at Minimal Combined Mass

A fundamental problem in biology is how cells organize their resource investment. Microbial metabolism, for example, typically involves hundreds of enzymes and metabolites, but it is unclear according to which principles their concentrations are set. Reasoning that natural selection will drive cells towards achieving a given physiological state at minimal cost, we derive a general equation that predicts the concentration of a metabolite from the concentration of the most abundant and costly enzyme consuming it. For effectively irreversible reactions, this relationship depends only on the enzyme's substrate affinity (K_m) and on the molecular cost ratio of enzyme and metabolite.

Simulations of cellular growth as well as experimental data demonstrate that relative costs are approximately proportional to molecular masses. The resulting model predicts *in vivo* metabolite concentration from enzyme concentrations with high accuracy across data from *E. coli* and diverse eukaryotes (R^2=0.85, mean fold-error 1.63), without fitting any free parameters. The corresponding organizing principle – the minimization of the summed mass concentrations of solutes – may facilitate reducing the complexity of kinetic models and will contribute to the design of more efficient synthetic cellular systems.

FEMS7-2661
The environmental virome

VIRUSES FROM HYPERSALINE ENVIRONMENTS

J. Antonio[1], M.D. Ramos-Barbero[1], T. Viver[2], J. Villamor[1], S. Fernando[1], M.G. Manuel[1], K. Konstantinos T.[3], R.M. Ramon[2]
[1]University of Alicante, Physiology- Genetics and Microbiology, Alicante, Spain
[2]IMEDEA, Marine Microbiology Group, Esporles, Spain
[3]Georgia Institute of Technology, School of Biological Sciences, Atlanta, USA

Hypersaline environments harbor the highest virus concentrations reported for aquatic systems as well as abundant prokaryotic communities with a relatively low diversity albeit a very high microdiversity, often explained as a response to virus predation. Therefore, they offer a unique and amenable scenario to study virus-host interactions in nature, away from the bias of laboratory systems.

With different modifications and nuances, models describing virus-host interactions assume that viruses infect the most abundant host communities, in a *Kill-the-winner* manner. Taking profit of the conditions offered by hypersaline systems, we have developed an experimental setting to analyze the fate and dynamics of an abundant bacterial population (*the winner*) inoculated in a crystallizer pond inhabited by a mature and stable microbial community. The goal was to analyze not only the effect of viral lysis on the targeted population but the changes induced in the microbial and virus assemblages.

Using a multiphasic approach including microscopy, cell and virus metagenomics, metatranscriptomics and virus isolation, we have followed the dynamics of the introduced host (a strain of the abundant and cosmopolitan Bacteroidetes Salinibacter ruber that was isolated from the analyzed saltern 15 years ago but was absent at the time of inoculation) and the rest of the microbial community. Changes in the host population were accompanied by a bloom of its viruses, likely wide host viruses infecting other strains previously present in the pond, that changed their diversity throughout the experiment time, as unveiled by the comparison of their genomes retrieved by cultivation and metagenomics.

FEMS7-2720
The environmental virome

PHAGES RARELY ENCODE ANTIBIOTIC RESISTANCE GENES: A CAUTIONARY TALE FOR VIROMOLOGY

E. François[1], R. Simon[2], S. Matthew B[2], P. Marie-Agnès[3]
[1]CNRS, Biology, Clermont-Ferrand, France
[2]The Ohio State University, Department of Microbiology, Columbus, USA
[3]Micalis Institute- INRA, Microbiology, Jouy-en-Josas, France

Despite their importance in natural ecosystems, the functional potential of viruses is yet to be determined at a community scale. Viral metagenomes provide a way to capture this potential, yet the recurrent presence of cellular DNA within these metagenomes hampers their analysis.
Here, we developped various bioinformatic strategies to detect cellular DNA. Considering only viromes with no cellular DNA detected, a significant number of cellular metabolism genes was retrieved in these viromes, suggesting that the presence of auxiliary genes involved in various metabolic pathways within viral genomes is a general trend in the virosphere.
We also developped strategies to detect Antibiotic resistance genes (ARGs) in viromes, as it has been suggested recently that ARGs are frequently encoded in phage genomes. Reanalysis of available human- or mouse-associated viromes for ARGs and their genomic context suggested that bona fide ARG attributed to phages in viromes were previously overestimated and reassert that ARGs are rarely encoded in phages.

FEMS7-0880
The environmental virome

COMPARATIVE GENOMICS OF THE GIANT PANDORAVIRUSES

M. Legendre[1], P. Olivier[1], A. Chantal[1], C. Jean-Michel[1]
[1]IMM, IGS, Marseille, France

In the early 1990s, an Amoeba parasite was serendipitously discovered from the water samples of a cooling tower in Bradford (UK). It turned out that this microorganism, misinterpreted as an intracellular bacterium, harbored a characteristic icosahedral viral morphology. It was actually the first giant virus ever discovered, named Mimivirus. Since then, a lot of effort has been made to discover new families of giant viruses from various environments, either through metagenomics surveys, or by direct isolation using Acanthamoeba as bait. In 2013, a new family of completely different giant viruses, the Pandoraviruses, was successfully isolated from sediments in Chile and Australia. These viruses exhibit a large ovoid particle of 1x0.5µm and hold the record of the largest viral genome with up to 2.5 Mb, reaching that of small parasitic eukaryotes. Right after, Pithovirus and Mollivirus, the members of two other giant virus families were discovered in Siberian permafrost samples. When compared with each other, these giant viruses show very little overlap in their gene content, which makes it difficult to understand their mode of evolution. By sampling various environments and geographical locations we have discovered new Pandoraviruses. We now have 6 complete genomes in hand, which allow us to perform comparative genomics. Furthermore, we used a compendium of “omics” data (i.e. genomics, transcriptomics and proteomics) to thoroughly annotate those genomes and tentatively answer this question: What makes a Pandoravirus?

FEMS7-2925
The human microbiome: education, communication, collaboration

THE HUMAN MICROBIOME - A COLLABORATIVE VENTURE

J. Jass[1]
[1]*Örebro University, School of Science and Technology, Örebro, Sweden*

Evidence has shown that the human microbiome has significant impact on health and disease. The human microbiome is the sum of all the microbial communities in association with different niches of the human body. It consists of diverse microbiota, including micro-eukaryotes and prokaryotes as well as viruses, with concerted physiological properties and is in balance with our own tissues. Thus the human microbiome has been considered a separate virtual organ. It is yet unclear what defines a healthy or diseased microbiome and how it may be manipulated to our benefit. Individual host and microbiota diversity and other compounding factors make it difficult to attribute specific features to improving health and reducing the risk of disease. The foundation of microbiome research is metagenomics, however, the advancement of other omics' methods such as metabolomics, and the ability to cultivate previously un-culturable microbes has allowed more complex structure –function analysis. The challenges that microbiome research faces today include the genetic diversity of human population, the functional diversity of the microbial communities, nutrition, diet, gender and lifestyle. This complexity is compounded by the large data sets that are often not comparable due to a lack of standardized approaches, and the inadequate tools for analysis. Thus there is a need for the development of innovative tools and increased training in handling and interpreting microbiome data. To improve our understanding of the human microbiome it is essential to develop multi-national and multi-disciplinary collaborative ventures that will generate novel research strategies.

FEMS7-3313
The human microbiome: education, communication, collaboration

I, SUPERORGANISM
J. Turney[1]
[1]*not applicable, not applicable, Bristol, United Kingdom*

I wrote one of the first books in English about the human microbiome. The topic poses interesting problems for the (would-be) popular writer. How to convey useful things about a world that is, normally, invisible? How to avoid giving unwarranted support to monocausal explanation and simple mechanisms - that is, being true to what we actually know about cell biology and the complexity of human systems? How to do that without simply repeating on every other page: it is all extremely complicated!? And finally, how to choose helpful metaphors in a domain that has not yet established any very helpful public discourse, and indicate that they *are* metaphors?

Many of these challenges are common to science writing about other subjects. I'll discuss whether they are harder to solve for this topic, and how one might try to do that as science communication about the microbiome develops.

FEMS7-1340
The human microbiome: education, communication, collaboration

THE HUMAN MICROBIOME: AN INTRODUCTION

A. Walker[1]
[1]*Rowett Institute, University of Aberdeen, Aberdeen, United Kingdom*

The human body is home to an extremely abundant and diverse collection of microbes. Indeed, some areas of the body, such as the large intestine, are colonised by some of the densest microbial communities found anywhere in nature.

The complexity of the human microbiome is truly humbling. Thousands of different species are capable of colonising the human body, and it has been estimated that our microbes encode more than 10 million unique genes (which is around 500 times more than the human genome). There is also huge microbiome variation between different individuals, and between bodily sites within the same individual.

Regardless of underlying microbiome variation, under normal circumstances our resident microbes are considered to play a number of key roles in the maintenance of human health. Conversely, the microbiome can also be a driver of disease. An emerging concept over the last decade has been that of “dysbiosis”, whereby the composition of the microbiota shifts from one that is generally benign or beneficial to host health to one that is deleterious for the host, and it is now clear that alterations in the microbiota are correlated with a whole range of diseases.

This has meant that public, academic and commercial interest in the microbiome has rocketed over the last decade. There is now a concerted effort, involving researchers around the world, to manipulate the microbiome for therapeutic purposes. There have been a number of encouraging advances, but much work remains to be carried out before we truly understand the role the microbiome plays, and how we might reproducibly alter it in beneficial ways.

A key challenge for educators, and those involved in knowledge exchange with the public, is to try and communicate exciting advances, while cutting through the hype that surrounds this field of research. In my talk I will give an overview of current knowledge, and provide some examples that may be useful as teaching resources.

FEMS7-3255
Yeast in action

HIGH THROUGHPUT CUSTOM ENGINEERING OF INDUSTRIAL YEAST STRAINS

E. Brevnova[1]
[1]*Ginkgo Bioworks, Organism Engineering, Boston, USA*

There is an emerging demand for sourcing plant-derived compounds (nutraceuticals, flavors, fragrances, sweeteners, etc.) from engineered microbes. While recent advances in synthetic biology and metabolic engineering provide feasible approaches to engineering such organisms, commercial success for developing these “cultured” ingredients presents specific challenges. Unlike biofuels, where efforts can be focused on one particular molecule given the enormous market size, cultured ingredients require developing different organism lines in a rapid and low cost fashion.

Scalable solution for bio-manufacturing of various organisms with multiple pathway engineered is provided by our state of the art foundry that continues to grow. Organism development at Ginkgo leverages our foundry to accelerate the design/build/test pipeline and empowered by combining computer-aided engineering software tools, cheap gene synthesis and high resolution-accurate analytical methods. Application of these approaches in combination allows rapid progress toward significant decrease of cultured ingredient production cost through strain and process optimization. Furthermore, improvements in manufacturing organisms lend to opportunities outside of cultured ingredients.

Examples of accelerated organism engineering of yeast industrial strains using Ginkgo technology will be demonstrated.

FEMS7-3326
Yeast in action

ENABLING THE PRODUCTION OF GLYCOSYLATED BIOPHARMACEUTICALS IN YEAST

N. Callewaert[1]
[1]*VIB and Ghent University, Center for Medical Biotechnology, Ghent, Belgium*

Glycan structures characterize the molecular environment immediately outside of all cell types and hence have critical functions in interactions of any cell with its environment (cell-cell, cell-pathogen, cell-molecule). The field of glycobiotechnology is concerned with understanding and re-engineering of these glycosylation-dominated interactions. In particular, the understanding of the synthetic pathways and functions for eukaryotic N- and O-glycosylation, gained over the past few decades, has enabled the rewiring of these pathways for the benefit of pharmaceutical applications. Based on the conservation of the core pathways between eukaryotes, it has been possible to transfer the efficient synthesis of particular human-specific glycan structures to other eukaryotes such as yeasts. This is enabling the cost-effective production of biopharmaceutical proteins with glycosylation patterns customized to particular therapeutic functionality (e.g. targeting to particular glycan receptors, or customized for particular pharmacokinetic behaviour). I will illustrate our work with regard to the production of human IgG-like glycosylation patterns in yeast (1), and the production of mannose-6-phosphate modified lysosomal enzymes for the treatment of human inherited lysosomal storage diseases (2). Whereas these synthetic biology endeavours were geared towards copying the synthesis of complex mammalian glycan structures in other eukaryotes, more recently we have generated cells (including yeast) in which glycosylation complexity has been reduced to the bare minimum, while still being compatible with eukaryotic cell life and protein productivity. This 'GlycoDelete' technology (3) opens up many new structural biology and biopharmaceutical applications.

1. Jacobs, P. P., Geysens, S., Vervecken, W., Contreras, R. & Callewaert, N. Engineering complex-type N-glycosylation in Pichia pastoris using GlycoSwitch technology. Nat. Protoc. 4, 58–70 (2009).

2. Tiels, P. et al. A bacterial glycosidase enables mannose-6-phosphate modification and improved cellular uptake of yeast-produced recombinant human lysosomal enzymes. Nat. Biotechnol. 30, 1225–1231 (2012).

3. Meuris, L. et al. GlycoDelete engineering of mammalian cells simplifies N-glycosylation of recombinant proteins. Nat. Biotechnol. 32, 485–489 (2014).

FEMS7-1967
Yeast in action

ENGINEERING OF LIPID METABOLISM IN SACCHAROMYCES CEREVISIAE

V. Siewers[1], *J. Nielsen*[1]
[1]*Chalmers University of Technology, Biology and Biological Engineering, Göteborg, Sweden*

Fatty acids serve as precursors for a number of industrially relevant molecule classes such as alkanes, alkenes, fatty alcohols and different types of esters including wax esters. These can be applied as biofuels, but also as ingredients in the food, cosmetics and pharmaceutical industry. Our aim is to develop sustainable production processes for these compounds through metabolic engineering of *Saccharomyces cerevisiae*.

In contrast to oleaginous yeasts, *S. cerevisiae* does not naturally accumulate large amounts of lipids. One approach is therefore the development of platform strains. This includes the streamlining of acetyl-CoA and acyl-CoA metabolism for increased precursor supply, but also efforts to modulate the fatty acid profile of these strains. A second approach is the screening of heterologous enzymes and pathways leading to the formation of the desired products.

FEMS7-0504
Anaerobic communities

STRATIFIED MICROBIAL COMMUNITIES IN THE OXIC-ANOXIC TRANSITIONS OF DEEP HYPERSALINE ANOXIC BASINS

D. Daffonchio[1]
[1]*KAUST- King Abdullah University of Science and Technology, BESE, Thuwal - Jeddah, Saudi Arabia*

The dissolution of ancient buried deep-sea evaporites by water penetrating fractures determined by tectonic movements has determined the formation of the so-called Deep Hypersaline Anoxic Basins (DHABs) in different oceanic regions. These brine pools are located at depths ranging from few hundreds of meters below sea level to almost 4000 meters such as some in the Mediterranean Sea. The chemical nature of the dissolved evaporites has largely driven the composition of the DHABs brines that present extreme conditions of high salinity, anoxia and generally a high content of hydrogen sulfide. The chemistry of the brines and the extreme conditions selected unique communities of microbial extremophiles with many novel unusual endemic phylotypes that do not have any cultivated representative. The most active component of these unique ecosystems is the transition zone between the oxic water column and the anoxic brines where the environmental conditions change in few meters depth with a sharp increase of salinity and density and dramatic increases and decreases of electron donors and acceptors concentrations. The sharp changes of redox couples along such chemoclines drive the stratification of microorganisms with different metabolisms and their coexistence in a narrow vertical portion of the water column. Here, the diversity and functional features of the stratified microbiomes in chemically different DHABs of the Red Sea is presented, individuating the functional network interactions within the stratified microbial assemblage.

FEMS7-3293
Anaerobic communities

ROLE OF BACTERIAL AND ARCHAEAL COMMUNITY MEMBERS INVOLVED IN ANAEROBIC OXIDATION OF METHANE

M. Jetten[1]
[1]*Soehngen Institute of Anaerobic Microbiology, Radboud University Nijmegen, Nijmegen, The Netherlands*

Anaerobic oxidation of ammonium by anammox bacteria or anaerobic oxidation of methane by M*ethylomirabils oxyfera* bacteria and *Methanoperedens* Archaea are recent discoveries in the nitrogen and methane cycle catalyzed by novel so-called impossible microbes [Strous et al 1999 Nature 400: 446-449; Raghoebarsing et al 2006 Nature 440: 918-921; Haroon et al 2013 Nature 501:578-581]. These anaerobic processes share many interesting aspects. Both anaerobic ammonium and methane oxidation processes were once deemed to be biochemically impossible and non-existent in nature, but have now been identified as important players in global nitrogen and methane cycling. Molecular studies showed that anammox bacteria can make the rocket fuel hydrazine by novel multiheme complexes [Kartal et al 2011 Nature 479: 127-130; Dietl et al Nature doi: 10.1038/nature15517] that are located in a unique bacterial organelle surrounded by ladderane lipids [van Niftrik & Jetten 2012 MMBR 76(3):585-596 doi: 10.1128/MMBR.05025-11]. The *M. oxyfera* bacteria turned out to have a new intra-aerobic metabolism. They are able to produce their own oxygen by conversion of 2 NO into O_2 and N_2 by a putative NO dismutase [Ettwig et al 2010 Nature 464: 543-548]. *Methanoperedens* Archaea can couple the reverse methanogenesis pathway to reduction of nitrate. Molecular surveys have indicated that these organisms are wide spread in anaerobic ecosystems around the globe where they most probably interact in an intricate anaerobic food chain. Furthermore all three microorganisms can be applied in sustainable, cost effective wastewater treatment for the removal of methane and nitrogen compounds and are investigated within the center of excellence in anaerobic microbiology (www.anaerobic-microbiology.eu) funded by the Netherlands Gravitation program 024.002.002 SIAM and ERC AG EcoMOM 339880.

FEMS7-2857
Anaerobic communities

HIGH-THROUGHPUT SEQUENCING ANALYSIS REVEALED MICROBE INTERACTIONS DURING PCB DETOXIFICATION IN CONTAMINATED MARINE SEDIMENTS

B. Matturro[1], *S. ROSSETTI*[1]
[1]*IRSA-CNR, WATER RESEARCH INSTITUTE, ROME, Italy*

Backgrounds

Polychclorinated biphenyls (PCBs) are among the most toxic contaminants widely found in several matrices including marine sediments. Anaerobic reductive dechlorination (RD) is the only bioprocess known to reduce such compounds to harmless or less toxic forms. Despite RD has been widely investigated, little is known about the identity and metabolic potentials of PCB-dechlorinating bacteria in real contaminated marine sediments.

Objectives

This study aimed to estimate the biodiversity and the bioremediation potential of PCB contaminated marine sediments.

Methods

The core microbiome of marine sediments taken from three different sites affected by PCB contamination was analysed by Next Generation Sequencing (NGS). Marine sediments were used to construct anaerobic microcosms with and without the addition of external electron donors to stimulate PCB reductive dechlorination.

Conclusions

The study revealed that the autochthonous bacteria were able to sustain the process using the sole sediment organic carbon. RD was observed in all microcosms and was particularly marked in the marine sediment affected by long-term contamination (up to 47% of PCB decrement was observed already after 70 days of anaerobic incubation).
Further, free-sediment microbial enrichments by using PCE as model contaminant were selected from all microcosms. They were mainly composed by > 80% of a novel non-*Dehalococcoides Chloroflexi* belonging to *Dehalococcoidia* class involved in the RD process.
Overall this study sheds light on the identity of both dechlorinating and flanking populations which support the RD process.

FEMS7-0770
Anaerobic communities

BACTERIAL COMMUNICATION ENTERS DIGITAL AGE -MEMBRANE VESICLES DELIVER OF QUORUM SENSING MOLECULE-

K. Morinaga[1], M. Toyofuku[1,2], J. Uhl[3], H. Shimamura[1], N. Obana[1], P. Schmitt-Kopplin[3,4], L. Eberl[2], N. Nomura[1]
[1]University of Tsukuba, Department of Life and Environmental Sciences, Tsukuba, Japan
[2]University of Zurich, Department of Plant and Microbial Biology, Zurich, Swaziland
[3]Helmholtz Zentrum München-German Research Center for Environmental Health, Research Unit Analytical BioGeoChemistry, Munich, Germany
[4]Technische Universität München, Chair of Analytical Food Chemistry, Munich, Germany

Backgrounds

Many Gram-negative bacteria use *N*-acyl-homoserine lactones (AHLs) as signal molecules to communicate with each other. The acyl side chains of AHLs contain 4 to 18 carbons, which are often additionally modified. Given the limited diffusibility of long chain AHLs it has remained a mystery how these signals are released from cells into aqueous environments.

Objectives

Paracoccus denitrificans produces *N*-hexadecanoyl-AHL (C16-HSL) that partitions with the cell membrane. Like many bacteria *P. denitrificans* can form membrane vesicles (MVs), which serve various biological functions. In this study, we investigated the role of MVs in C16-HSL based cell-cell communication.

Methods

MV was collected from cell culture by ultracentrifugation. C16-HSL delivery was monitored by using AHL reporter strains. In-frame gene deletion mutants were generated by homologous recombination. C16-HSL concentration was quantified by UHPLC-qToF-MS.

Conclusions

Our results show that C16-HSL was enriched in MVs and we determined that the AHL concentration contained by a single MV is high enough to trigger QS-regulated gene expression in a bacterial cell. The influence of MVs on C16-HSL-controlled cell aggregation in *P. denitrificans* was investigated and compared to free C16-HSL. In contrast to the conventional QS model in which free diffusion of signals is assumed, we propose a novel MV-based mechanism for binary trafficking of hydrophobic signal molecules, which may be particularly relevant for bacteria that live in open aqueous environments. Furthermore, MVs showed varying propensities to different bacteria, suggesting that the signals are targeted to certain cell types.

FEMS7-0166
Anaerobic communities

A BIOGEOCHEMICAL AND MOLECULAR PERSPECTIVE INTO AN ORGANIC SULFUR AND NITROGEN CYCLE INTERACTION

P. Salgado[1], R. Kiene[2], M.A. Moran[3], H. Ribeiro[1], C. Magalhães[1]
[1]Interdisciplinary Centre of Marine and Environmental Research - CIIMAR, EcoBioTec Laboratory, Matosinhos, Portugal
[2]University of South Alabama, Department of Marine Sciences, Mobile, USA
[3]University of Georgia, Department of Marine Sciences, Athens, USA

Backgrounds

Previous research has confirmed a novel interaction between the marine organic sulfur and nitrogen cycles, whereby methanethiol (MeSH), a dimethylsulfoniopropionate (DMSP) degradation intermediate, inhibits the final step of the denitrification pathway, limiting nitrogen loss through dinitrogen (N_2) and enhancing nitrogen release via N_2O, a potent greenhouse gas.

Objectives

The present study aimed to evaluate how MeSH regulates the N_2O reduction step of denitrification at the transcription level.

Methods

We investigated the effect of increasing MeSH additions on cell suspensions of *Ruegeria pomeroyi* DSS-3 and quantified the denitrification transcripts of *nirS*, *norB* and *nosZ* genes, encoding the nitrite, nitric oxide and nitrous oxide reductases respectively, through quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR). Also, we quantified the N_2O production in the MeSH treatments using an electron-capture detector (GC/ECD).

Conclusions

N_2O accumulation was exacerbated with increased MeSH concentrations, an expected pattern based on previous studies. The *nirS* and *norB* transcripts did not reveal significant differences in the different MeSH treatments. For the *nosZ* transcription signature, we expected to obtain an inversely proportional inhibition along the progressive MeSH additions, however we obtained the opposite pattern revealing an overexpression of the *nosZ* transcript. These results indicate that MeSH isn't inhibiting *nosZ* transcription but rather might be up-regulating it. The accumulation of N_2O after MeSH addition therefore may result from an inhibitory interaction at the protein level, interfering with functioning of the nitrous oxide reductase.

FEMS7-0380
Anaerobic communities

A NOVEL PATHWAY THAT LINKS THE SULFUR AND NITROGEN CYCLES: ANAEROBIC OXIDATION OF ELEMENTAL SULFUR COUPLED TO RESPIRATORY AMMONIFICATION OF NITRATE

G. Slobodkina[1], A. Mardanov[2], N. Ravin[2], A. Frolova[1], N. Chernyh[1], E. Bonch-Osmolovskaya[1], A. Slobodkin[1]
[1]Winogradsky Institute of Microbiology,
Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia
[2]Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

Backgrounds

In many natural and anthropogenic environments, biogeochemical cycles of nitrogen and sulfur are linked due to the activity of autotrophic microorganisms. However to date, sulfur-dependent dissimilatory nitrate reduction to ammonium has been demonstrated only with sulfide as an electron donor. Respiratory ammonification of nitrate (also known as dissimilatory nitrate reduction to ammonium, DNRA) is the microbial process that determines the retention of nitrogen in an ecosystem.

Objectives

We detected a novel pathway that couples the sulfur and nitrogen cycles.

Methods

Thermophilic anaerobic bacteria *Thermosulfurimonas dismutans* and *Dissulfuribacter thermophilus*, isolated from deep-sea hydrothermal vents, grew autotrophically with elemental sulfur as an electron donor and nitrate as an electron acceptor producing sulfate and ammonium. The genomes of both bacteria contain a gene cluster that encodes a putative nitrate ammonification enzyme system. Nitrate reduction occurs via a Nap-type complex. The reduction of produced nitrite to ammonium does not proceed via the canonical Nrf system because nitrite reductase NrfA is absent in the genomes of both microorganisms. The genomes of *D. thermophilus* and *T. dismutans* encodes a complete sulfate reduction pathway, while the Sox sulfur oxidation system is missing in both bacteria.

Conclusions

Thus, in high-temperature environments, nitrate ammonification with elemental sulfur may represent an unrecognized route of primary biomass production. Moreover, the anaerobic oxidation of sulfur compounds coupled to growth has not previously been demonstrated for the members of *Thermodesulfobacteria* or *Deltaproteobacteria*, which were considered exclusively as participants of the reductive branch of the sulfur cycle.

FEMS7-1506
Bacterial cell imaging

ESCHERICHIA COLI FTSA ASSEMBLES INTO POLYMERIC RINGS ON MEMBRANES THAT ALIGN FTSZ PROTOFILAMENTS AND ANTAGONIZE THEIR BUNDLING

M. Krupka[1], V. W. Rowlett[1], D. Morado[2], H. Vitrac[3], K. Schoenemann[1], J. Liu[2], W. Margolin[1]
[1]The University of Texas McGovern Medical School, Microbiology and Molecular Genetics, Houston, USA
[2]The University of Texas McGovern Medical School, Pathology and Laboratory Medicine, Houston, USA
[3]The University of Texas McGovern Medical School, Biochemistry and Molecular Biology, Houston, USA

Backgrounds

Bacteria such as *Escherichia coli* divide using a protein machine called the divisome that spans the cytoplasmic membrane. Key divisome proteins on the cytoplasmic side of the membrane include the tubulin-like **FtsZ**, which forms GTP-dependent protofilaments, and the actin-like **FtsA**, which tethers FtsZ to the membrane. ZipA and the **Zap** proteins act to **bundle FtsZ protofilaments** and join FtsA and FtsZ to form a septal ring.

Objectives

An FtsZ gain-of-function mutant, FtsZ* enhances FtsZ polymer bundling *in vitro* and is resistant to excess FtsA, which is normally toxic for cells. This prompted us to hypothesize a **novel role for FtsA** as an inhibitor of FtsZ protofilament bundling.

Methods

To provide genetic evidence that FtsA antagonizes FtsZ protofilament bundling *in vivo*, we overproduced FtsA in FtsZ overbundling and underbundling conditions, by overexpressing and deleting the *zap* genes, respectively. To demonstrate this bundling inhibition *in vitro* we employed a **lipid monolayer assay** followed by electron microscopy and 3D tomography analysis.

Conclusions

FtsA overproduction antagonizes the FtsZ overbundling triggered by ZapA protein and exacerbates the *zap* deletion phenotype. Unlike FtsA proteins from other bacterial species that form long polymers *in vitro*, ***E. coli* FtsA strikingly assembles into arrays of minirings** on lipid monolayers. These FtsA minirings promote FtsZ assembly into long, often parallel protofilaments and **prevent them from bundling**. These *in vitro* data support our *in vivo* evidence that FtsA antagonizes FtsZ protofilament bundling, and suggest that the oligomeric state of FtsA may influence FtsZ higher order structure and divisome function.

FEMS7-2058
Bacterial cell imaging

CELL WALL PROTEINS INVOLVED IN DIFFERENTIAION AND CELL-CELL COMMUNICATION IN MULTICELLULAR CYANOBACTERIA

I. Maldener[1], *J. Bornikoel*[1], *E. Bok*[1], *K. Forchhammer*[1]
[1]*Interfaculty Institute of Microbiology and Infection Medicine, Microbiology/Organismic Interactions, Tübingen, Germany*

Backgrounds

In order to differentiate specialized cells, the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120 processes its peptidoglycan in the intercellular septa by forming semi-regular arranged nanopores. This nanopore array allows the formation of septal junctions for cell-cell communication and is necessary for cell differentiation. Previously we showed that the cell wall lytic protein AmiC2 of *Nostoc punctiforme*, a homologue of cell division protein AmiC from *E. coli,* is involved in nanopore formation.

Objectives

We performed a functional study of the homologues of NlpD (the activator of AmiC in *E. coli*) and AmiC1 from *Anabaena* to investigate their role in cell-cell communication, nanopore array formation and differentiation of functional heterocysts, and hence diazotrophic growth. To study the cell wall modification on molecular level, we investigated the chemical composition and the structure of the peptidoglycan of *Anabaena* wild-type and cell wall mutants.

Methods

Creation of mutants
Fluorescence microscopy
Electron microscopy
FRAP studies
Sacculi isolation
UPLC
MS

Conclusions

Mutants in *nlpD1* and *amiC1 are* strongly hampered in nanopore formation and cell-cell communication. Even they differentiate heterocysts and show low N_2 fixation rates, they cannot grow diazotrophically, possibly because they cannot transfer the fixed nitrogen compounds. This confirms the importance of nanopore formation by these cell wall enzymes and the role of septal junctions in metabolite exchange. In conclusion we could show that the peptidoglycan of *Anabaena* exhibits unique features and represents a dynamic structure. It influences vital processes in the complex life style of multicellular cyanobacteria and is important for the survival in changing environments.

FEMS7-0095
Bacterial cell imaging

THE TORTOISE AND THE HARE: A ROLE FOR CONSTRICTION IN CELL SIZE HOMEOSTASIS

S. Manley[1], A. Lambert[1], A. Vanhecke[1], S. Holden[2], A. Archetti[1], F. Schaber[1], E. Goley[3]
[1]Ecole Polytechnique Federale de Lausanne EPFL, Institute of Physics, Lausanne, Switzerland
[2]Newcastle University, Centre for Bacterial Cell Biology- Institute for Cell and Molecular Biosciences, Newcastle, United Kingdom
[3]Johns Hopkins University School of Medecine, Department of Biological Chemistry, Baltimore, USA

Super-resolution fluorescence microscopy offers new insights into the subcellular organization and cell-cycle mechanics of bacteria. Previously, we have shown that with automation and optimized illumination, we can obtain large statistics on bacteria shape and protein organization over the cell cycle.

Underlying bacterial cell size homeostasis is the "adder rule": a constant addition of length or volume per cell cycle. However, its mechanism is poorly understood. We study the enforcement of this rule by dissecting single cell shape dynamics with time-lapse structured illumination microscopy (SIM). Examining a hyperactive constriction mutant of septal peptidoglycan remodeling (FtsWI*), and treatment with a peptidoglycan synthesis inhibitor (fosfomycin), we find that onset and duration of constriction together determine elongation.**

FEMS7-0245
Bacterial cell imaging

MEMBRANE VESICLE FORMATION THROUGH CELL DEATH

M. Toyofuku[1], *N. Nomura*[1], *L. Eberl*[2]
[1]*University of Tsukuba, Life and Environmental Sciences, Tsukuba, Japan*
[2]*University of Zurich, Department of Plant and Microbial Biology, Zurich, Switzerland*

Backgrounds

Membrane vesicles (MVs) that are released by both Gram-negative and Gram-positive bacteria are involved in many biological processes, including horizontal gene transfer, virulence, phage decoy and cell-to-cell communication, and also have impact on human health. Despite their biological importance, little is known about the mechanisms of MV biogenesis.

Objectives

Our study aimed at understanding how MVs are produced in bacteria using *Pseudomonas aeruginosa* and *Bacillus subtilis* as model organisms.

Methods

MVs were isolated by ultracentrifugation and quantified with membrane specific fluorescent dyes. RNA associated with MVs was sequenced to gain insight of the genes involved in MV formation. To understand the procedure of MV biogenesis in detail, we used different live cell imaging techniques.

Conclusions

Live cell imaging showed that a subpopulation of the cells undergo explosive cell lysis and generate MVs in *P. aeruginosa*. RNA-seq of MV-associated RNA revealed that genes related to stress response were enriched in MVs. Among these genes, an endolysin gene was found to be critical to trigger explosive cell lysis. Endolysins are conserved among bacteria and are typically related to phage or bacteriocin release. Likewise, an endolysin was also found to be involved in MV formation of *B. subtilis*. In contrast to explosive cell lysis in *P. aeruginosa*, endolysin-triggered MV formation in *B. subtilis* proceeded through a pinhole in the cell wall that allowed the extrusion of membrane material (named bubbling cell death). Our results show that the widely distributed endolysin genes triggers MV formation through cell death in both Gram-positive and -negative bacteria, albeit *via* different mechanisms.

FEMS7-0877
Bacterial cell imaging

POLAR INSERTION OF THE OUTER MEMBRANE COMPONENTS AND HETEROGENEITY OF THE OUTER MEMBRANE OF BRUCELLA ABORTUS

V. Vassen[1], C. Formosa-Dague[2], C. Valotteau[2], C. Feuillie[2], N. Auberger[3], S. Vincent[3], Y. Dufrêne[2], J.J. Letesson[1], X. De Bolle[1]
[1]University of Namur, Research Unit in Biology of Microorganisms, Namur, Belgium
[2]Catholic University of Louvain, Institute of Life Sciences, Louvain-la-Neuve, Belgium
[3]University of Namur, Bio-Organic Chemistry Laboratory, Namur, Belgium

Backgrounds

Brucella abortus is an α-proteobacteria and one of the etiological agents of brucellosis, a worldwide-spread zoonosis. Bacteria belonging to the order Rhizobiales are characterized by unipolar growth. Labeling of envelope using Texas red succinimidyl ester (TRSE) revealed the new pole and the constriction site as proposed growth sites in *B. abortus*.

Objectives

Since the new material is incorporated at the new growing pole and the constriction site, we are interested in the insertion of the different layers of the bacterial envelope: lipopolysaccharide (LPS), outer membrane proteins (Omp) and peptidoglycan (PG).
Moreover, the general organization of the outer membrane with regard to its heterogeneity will be studied.

Methods

B. abortus 544 was fully labeled with monoclonal antibodies directed against smooth (S)-LPS or Omp25 or fluorescent D-amino acid HCC-amino-D-alanine (HADA) and absence of these signals after growth corresponds to newly incorporated materials.
Additionally, the insertion of new LPS was shown using a rough (R) strain in which the synthesis of O-chain, and thus S-LPS, was inducible. Furthermore, the structure of the outer membrane of fixed bacteria was examined by using Atomic Force Microscopy (AFM).

Conclusions

Labeling experiments showed that new S-LPS, Omp25 as well as PG are incorporated at the proposed growth sites, i.e. the new pole and the constriction site. Moreover, none of these structures seems to be mobile under the conditions in which they were observed.
Study of the outer membrane by AFM confirmed preliminary data of the appearance of R-LPS clusters on the wild type cells.

FEMS7-2514
Bioactive metabolites

A MINIMAL MODEL OF PHOTOTROPHIC GROWTH TO EXPLAIN PROTEOME ALLOCATION IN CYANOBACTERIA

M. Faizi[1], *R. Steuer*[1]
[1]*Institute for Theoretical Biology, Department of Biology- Humboldt University of Berlin, Berlin, Germany*

Backgrounds

Cellular metabolism must continuously adapt to a multitude of changing environmental conditions to ensure best possible growth. The need for continuous adaptation is in particular true for phototrophic organisms that rely on harvesting the sun's energy to survive and grow. Phototrophic metabolism is assumed to be carefully orchestrated to meet cellular demands for energy requirements, to avoid photodamage, and to provide sufficient storage compounds for periods of darkness.

Objectives

Our focus is to understand the underlying mechanisms of phototrophic growth and the adaptive properties of cellular metabolism. Recent studies on heterotrophic bacterial growth investigated the implications of cellular proteome allocation on cellular growth. That is, how do cells allocate their finite resources to different cellular processes, and how does this allocation impact metabolism and growth?

Methods

Current mathematical descriptions of bacterial growth show that the relationships that govern optimal resource allocation can be derived from coarse-grained kinetic models. Here, we present a minimal dynamic model of phototrophic growth that adapts its coarse-grained proteome to different light and CO_2 conditions.

Conclusions

The model predicts relationships that govern optimal resource allocation of phototrophic growth and is able to reproduce cyanobacterial growth laws based on minimal assumptions about key parameters.

FEMS7-2457
Bioactive metabolites

BACTERIAL SECONDARY METABOLITE PRODUCTION VIA HETEROLOGOUS GENE CLUSTER EXPRESSION IN PSEUDOMONAS PUTIDA

A. Loeschcke[1], *A. Domröse*[1], *R. Weihmann*[1], *K.E. Jaeger*[1], *T. Drepper*[1]
[1]*Heinrich-Heine-University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany*

Backgrounds

The wealth of bacterial secondary metabolites includes numerous compounds with relevant bioactivities such as antibiosis or cytotoxicity.

Objectives

To enable biotechnological access *via* heterologous biosynthesis in an amenable host, cloning of the relevant genes, their transfer, stable maintenance and functional expression has to be accomplished. Moreover, the host cell metabolism is required to provide a suitable metabolic background for establishing a biosynthetic pathway. Therefore, effective genetic tools are required that allow the effective cloning and heterologous expression of large gene clusters in diverse bacterial hosts.

Methods

We have developed the molecular genetic tools TREX and yTREX, that consist of gene cassettes enabling i) the straightforward yeast recombinational cloning of large gene clusters, ii) the conjugational transfer of a TREX-labeled gene cluster into bacterial hosts and iii) its stable integration into the host chromosome *via* transposition. iv) Finally, expression of the gene cluster can be realized by random integration downstream of a chromosomal promoter or by employment of convergent T7 RNA polymerase-mediated expression.

Conclusions

We could demonstrate several key benefits of the TREX tools. Straightforward one-step recombination in yeast enabled rapid cloning of several gene clusters, including carotenoid, prodigiosin, violacein and phenazine biosynthesis clusters that range in size from 6 to 21 kb. Using *Pseudomonas putida* as host, random genomic gene cluster integration enabled identification of naturally highly transcribed chromosomal regions suitable for gene cluster expression, and metabolite production at mg scale. The presented tools thus offer new perspectives in the fields of genome mining and synthetic biology.

FEMS7-2070
Bioactive metabolites

ANTIMICROBIAL ACTIVITY OF PEPTIDES DERIVED FROM LYTA AUTOLYSIN AGAINS STREPTOCOCCUS PNEUMONIAE

E. Roig[1], *M. Beatriz*[1], *S. Manuel*[2], *S. Jesús Miguel*[1]
[1]*Universidad Miguel Hernández, Instituto de Biología Molecular y Celular, Elche Alicante, Spain*
[2]*Universidad Miguel Hernández, Dpt. Vegetal Production and Microbiology, Elche Alicante, Spain*

Backgrounds

Streptococcus pneumoniae (pneumococcus) is one of the major bacterial pathogens that causes several diseases with high social impact such as pneumonia, meningitis and otitis media. Vaccination and antibiotic treatment are two strategies to combat this pathogen, which are incomplete due to the high serotype variety and the increasing antibiotic resistance, so that the search and development of new antimicrobials are necessary.

Objectives

Choline-binding proteins (CBPs) of *S. pneumoniae* are an attractive target for antimicrobials because they are essential for bacterial viability and virulence, and are common to all serotypes. All CBPs are composed of a choline-binging module (CBMs) which consist of repetitive motifs (CBRs, choline-binding repeats), rich in aromatic amino acids. It has been reported that C-LytA (CBMs from the LytA amidase) in vitro competes with their parental cell-wall hydrolases and inhibit their function. Taking advantage of the fact that a single CBR (239-252 of LytA) acquires a stable conformation in solution we have studied the structure and the antimicrobial effect of this peptide and its derivatives obtained from the duplication and triplication of sequence.

Methods

The structure of CBR-derived synthetic peptides was studied by circular dichroism. Then we performed viability assays of pneumococcal cultures treated with these peptides. A clear enhancement in antimicrobial effect was observed as the number of peptide sequence repeats was increased.

Conclusions

We have unveiled the structural determinants of peptides derived from CBRs for the design of new antipneumococcal drugs.

FEMS7-2214
Cell surfaces and signal transduction

NITROGEN FIXING SYMBIOSIS AND BACTERIAL CELL CYCLE ARE LINKED IN SINORHIZOBIUM MELILOTI

S. Xue[1], E. Biondi[1]
[1]IMM, LCB, Marseille, France

Backgrounds

In all domains of life, proper regulation of the cell cycle is critical to coordinate genome replication, segregation and cell division. In some groups of bacteria, *e.g. Alphaproteobacteria,* tight regulation of the cell cycle is also necessary for the morphological and functional differentiation of cells. During the symbiosis in *Medicago* species, the alphaproteobacterium *Sinorhizobium meliloti* undergoes an elaborate cellular differentiation within host root cells. This differentiation results in massive amplification of the genome, cell branching and elongation, and loss of reproductive capacity.

Objectives

In the closely related alphaproteobacterium *Caulobacter crescentus*, cellular differentiation is tightly linked to the cell cycle via the activity of the master regulator CtrA, and recent research in *S. meliloti* suggested that CtrA might also be key to cellular differentiation during symbiosis. Depletion of CtrA causes cell elongation, branching and genome amplification, similar to that observed in nitrogen-fixing bacteroids.

Methods

We showed that the cell cycle regulated proteolytic degradation of CtrA is essential in *S. meliloti* and all mutants of the CtrA degradosome are impaired in the differentiation process. As CtrA must be absent in mature bacteroids allowing a proper nitogen fixation we hypothesized that CtrA proteolysis may play a crucial role in bacteroid development and possibly triggered by NCR plant peptides.

Conclusions

Our findings provide valuable insight into how highly conserved genetic networks can evolve, possibly allowing complex differentiation programs such as the bacteroid differentiation in *S. meliloti.*

FEMS7-0035
Cell surfaces and signal transduction

FOLLOWING COLICIN TRANSLOCATION TO THE ESCHERICHIA COLI CYTOPLASM ONE MOLECULE AT A TIME

M.L. Francis[1], *P. Rassam*[1], *C. Kleanthous*[1]
[1]*University of Oxford, Biochemistry, Oxford, United Kingdom*

Backgrounds

Gram-negative bacteria often release toxic bacteriocins into their immediate environment as a means of competing with closely related microorganisms. Colicins are potent bacteriocins expressed and released by *E. coli* that bind to outer membrane protein receptors with high affinity and, following contact with proteins in the periplasm and inner membrane, translocate a cytotoxic domain into the cell by a poorly understood mechanism. Cell-death occurs in minutes through depolarization of the cell, hydrolysis of peptidoglycan precursors or digestion of nucleic acids in the cytoplasm. The dogma in colicin research is that a single molecule is sufficient to kill a cell, although this has yet to be formerly demonstrated.

Objectives

The aim of this research was to develop fluorescence-based microscopy tools to visualise colicin entry into *E. coli* as a test of this hypothesis and to probe the mechanism of translocation through the cell envelope.

Methods

We have developed fluorescence widefield and total internal reflection fluorescence (TIRF) microscopy methods for investigating the import of single colicin E9 molecules in *E. coli*.

Conclusions

We demonstrate that ColE9 fluorescently-labelled in its C-terminal endonuclease domain retains catalytic activity and cytotoxicity. Using this fluorescently-labelled ColE9 we have for the first time visualised the translocation of individual colicin molecules to the cytoplasm of *E. coli*, tracked the diffusion of these single molecules *in vivo* and demonstrated that import is dependent on the proton motive force. This research will describe current progress in exploiting these newly developed tools in understanding bacteriocin translocation in bacteria.

FEMS7-2867
Cell surfaces and signal transduction

MICROBES, METALS AND NANOWIRES

G. Reguera[1]
[1]*Michigan State University, Microbiology and Molecular Genetics, East Lansing, USA*

Backgrounds

In Nature, highly efficient and diverse consortia of microbes cycle carbon and other elements in electrochemical reactions that discharge metabolic electrons onto soluble and insoluble metals. One group in particular, *Geobacter* bacteria, uses nanoscale, conductive protein filaments or pili to bind and transfer electrons to iron oxide metals and to soluble metal contaminants such as the uranyl cation, which is reductively precipitated outside the cell. Interestingly, the *Geobacter* pili are composed of a single peptide subunit, which polymerizes in a helical fashion such that contacts are formed between the side chains of aromatic residues to promote fast rates of charge hopping. Furthermore, structural studies identified ligands on the pilus surface that could function as metal traps for the uranyl cation and other cationic metals. Once bound, the metals are positioned in close proximity to a terminal tyrosine residue of the pilus multistep hopping path to promote their reductive precipitation.

Objectives

To investigate protein nanowires as a new paradigm in biological electron transfer
To harness the properties of conductive nanobiomaterials in nanotechnological applications

Methods

Methods interface micro fabrication, atomic force microscopy and biological techniques to study protein nanowires and integrate them into electronic devices.

Conclusions

The nanoscale dimensions of the pili and their ability to transport charges and immobilize metals offer unique opportunities for applications in nanotechnology. On the other hand, recombinant techniques can be applied to develop sustainable manufacturing protocols for the mass-production of peptides and protein nanowires. Further, genetic engineering can be used to functionalize the nanomaterials for their selective and specific integration into nanoelectronic devices. Of special significance is the ability of these nanomaterials to bind and reductively precipitate metal contaminants such as uranium, a process that could be harnessed to develop sensors and deployable devices for bioremediation.

FEMS7-0606
Cell surfaces and signal transduction

THE LIPOPROTEIN EXPORT SIGNAL OF BACTEROIDETES

F. Lauber[1], G.R. Cornelis[1], F. Renzi[1]
[1]UNamur, Biology, Namur, Belgium

Backgrounds

The phylum Bacteroidetes includes human pathogens and commensals as several members of the human microbiota that contribute to the control of gut homeostasis and protection against pathogens. A hallmark of these bacteria is the presence of surface-exposed multi-protein membrane complexes (Sus-like systems) that are encoded by specific polysaccharide utilization loci (PUL). These systems are unique and have a crucial role in the biology of Bacteroidetes since they allow the uptake and catabolism of a large variety of nutrients, mainly polysaccharides coming from the host. Sus-like systems are mainly composed of lipoproteins anchored to the outer membrane and facing the external milieu. This lipoprotein localization is uncommon in most studied Gram-negative bacteria while it is widespread in Bacteroidetes.

Objectives

Little is known on how these complexes assemble and in particular on how lipoproteins are transported at the bacterial surface.

Methods

By bioinformatic analyses, we identify a lipoprotein export signal (LES) at the N-terminus of surface-exposed lipoproteins of the human pathogen *Capnocytophaga canimorsus* corresponding to K-$(D/E)_2$ or Q-A-$(D/E)_2$. We show that, when introduced in sialidase SiaC, an intracellular lipoprotein, this signal is sufficient to target the protein to the cell surface. Mutational analysis of the LES in this reporter system showed that the amino acid composition, the position of the signal sequence and the global charge are critical for lipoprotein surface transport. Furthermore, we identify a LES in other Bacteroidetes species.

Conclusions

Overall these findings suggest the presence of a common new bacterial lipoprotein export pathway that flips lipoproteins across the outer membrane of Bacteroidetes.

FEMS7-2877
Cell surfaces and signal transduction

BUILDING A MEMBRANE ON THE OTHER SIDE OF THE WALL
T.J. Silhavy[1]
[1]*Princeton University, Molecular Biology, Princeton, USA*

Building a membrane on the other side of the wall

Thomas J. Silhavy

Princeton University

The outer membrane (OM) of Gram-negative bacteria functions as a protective barrier. It is unusual because the OM bilayer is asymmetric; the inner leaflet is composed of phospholipids (PLs), but the outer leaflet is made of lipopolysaccharide (LPS). Two kinds of proteins are found in the OM. Lipoproteins (Lpps) are inserted into the inner leaflet of the OM by posttranslationally attached lipid moieties. Integral OM proteins are β-barrel proteins (OMPs). I will present an historical overview of how the structure of the Gram-negative cell envelope was discovered and then summarize what is currently known about the assembly pathways for LPS, Lpps and OMPs in *Escherichia coli*. What's missing is information about the assembly pathway for phospholipids (PLs).

Several years ago we discovered a retrograde PL transport system that function to maintain OM asymmetry. I will describe a dominant mutation in the gene for the OM lipoprotein component of this transport system that leads to cell death under starvation conditions in media with limited cation concentrations. Death occurs not by rapid cell lysis, but by a novel mechanism involving flow of PLs from the inner to the OM that results in rupture of the inner membrane and the slow leakage of cytoplasmic contents. Our data may provide insights into the long-standing question of how PLs are transported to the OM.

FEMS7-3292
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

SMALL WORLD INITIATIVE (SWI)
N. Broderick[1]
[1]*Molecular and Cell Biology, University of Connecticut, USA*

The Small World Initiative is a novel introductory research course that addresses a human health crisis – the need for new antibiotics. Launched by Dr. Jo Handelsman at Yale University in 2012 and piloted by 26 institutions in 2013, the program has rapidly expanded to 185 institutions across 12 countries. Through the course, students: a) discover and characterize antibiotic-producing bacteria from soil, b) extract their metabolites, and c) contribute their data with student researchers from around the globe. This initiative integrates two critical elements. First, it provides an early discovery-based research experience for students, which encourages STEM retention. Second, the course seeks to positively impact human health by crowdsourcing the discovery of new antibiotics. Recently, SWI has expanded to high schools in the US, UK, Ireland, and Spain. While we continue to grow these programs, we are developing future phases: establishing throughput and educational chemical labs to move leads into the drug discovery pipeline, and additional professional development opportunities for faculty and students. The next phase of the program is well poised and we look forward to the addition of new collaborators to join our mission and promote antibiotic discovery through the curiosity and creativity of young scientists across the world.

FEMS7-1209
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

A LABORATORY ACTIVITY USING BACTERIOPHAGES, THE FORGOTTEN WEAPON AGAINST BACTERIA

S. Campoy[1], *C. Pilar*[1], *J. Aranda*[1], *M. Sánchez-Osuna*[1], *J. Barbe*[1], *M. Llagostera*[1]
[1]*Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, Cerdanyola del Vallès, Spain*

Backgrounds

Soon after their discovery, 100 years ago, phages were used to treat patients suffering from dysentery or cholera and to promote wound recovery. Although the results were promising, interest in phage therapy waned until it was mostly forgotten, as penicillin and other antibiotics became widely available. Today, the emergence of multidrug-resistant bacteria has rekindled the interest in bacteriophages as antibacterial tools. Despite phages are the most divers and abundant entities in our planet and their multiple applications, biology students remain largely unaware of these entities and are offered few opportunities to explore them in the laboratory.

Objectives

In this activity, students are introduced to the discovery of bacteriophages and encouraged to design an experiment demonstrating the possible use of lytic phages as biocontrol agents and comparing their efficacy with that of other antimicrobial agents. Further, they also formed hypotheses about the expected results and then, using quantitative data, tested their validity.

Methods

Experimental data were obtained by measuring absorbance and cell viability of *Salmonella enterica* sv Typhimurium LT2 cultures treated with either the P22 lytic derivative bacteriophage or different antibiotics (streptomycin, ampicillin or spectinomycin).

Conclusions

The obtained data, combined with class data or student autonomous learning, contributed to providing insights into the lytic and lysogenic cycles of bacteriophages, the molecular processes that finally kill the bacteria, and the appearance resistant bacteria. Further, using the scientific method, students were able to explore the nature of antimicrobial agents and the possible use of phages, phage cocktails, and phage derivative compounds as antibacterial tools.

FEMS7-0904
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

FIRST RESULTS OF THE EXPERIENCE IN MADRID AND FUTURE PERSPECTIVES

V.J. Cid[1], P. Calvo[2], R. Díez-Orejas[1], T. Fernández-Acero[1], J. Gil-Serna[2], B. González-Zorn[3], L. de Juan[4], H. Martín[1], M. Molina[1], F. Navarro-García[1], B. Patiño[2], J. Pla[1], D. Prieto[1], C. Rodríguez[1], E. Román[1], M.I. de Silóniz[2], M. Suárez[3], M.J. Valderrama[2], C. Vázquez[2]
[1]Universidad Complutense de Madrid, Departamento de Microbiología II- Facultad de Farmacia, Madrid, Spain
[2]Universidad Complutense de Madrid, Departamento de Microbiología III- Facultad de Biología, Madrid, Spain
[3]Universidad Complutense de Madrid, Departamento de Sanidad Animal- Facultad de Veterinaria, Madrid, Spain
[4]Universidad Complutense de Madrid,
Departamento de Sanidad Animal y VISAVET- Facultad de Veterinaria, Madrid, Spain

Backgrounds

WHO considers Antimicrobial Resistance (AMR) among the greatest threats to human health. Therefore, education in AMR awareness currently stands as a major priority in scientific communication. The Small World Initiative (SWI) is an educational crowdsourcing programme created in the USA in 2012: college students perform both field and lab work to isolate novel bacterial strains with antimicrobial bioactivities from environmental samples. Involving students in a real discovery experience inspires young students to pursue scientific careers and successfully spreads AMR awareness.

Objectives

Our objective was adapt SWI to the particular educational environment of Spain, in which curricular decisions are taken early in academic life, essentially at the High School level. At the same time, we wanted to involve Microbiology students at the University, bridging the gap between Higher and Secondary Education.

Methods

We adopted a service-learning strategy: teams of 4-6 volunteer University students, supervised by 19 Microbiology Faculty members, creatively implemented SWI on groups of 10-30 students in High Schools in the region of Madrid. A total 130 College students from 6 different Degrees worked on 22 local High Schools, where over 500 students collected and analyzed 250 soil samples. Blogs and social networks were exploited to communicate the experience and surveys were used to assess its impact at diverse levels.

Conclusions

We present our encouraging conclusions on how the pilot SWI@Spain experience contributed to (i) raising AMR awareness in our community; (ii) the formation of University students by an alternative service-learning pedagogical methodology; (iii) the motivation of High School students towards science-oriented University degrees.

FEMS7-0601
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

SIMPLE PROTOCOL FOR MOLECULAR FINGERPRINTING OF HUMAN ORAL MICROBIOTA SAMPLES IN LAB CLASSES

A.C. Henriques[1], P. De Marco[1]
[1]IUCS, IINFACTS, Paredes, Portugal

Backgrounds

The increasing significance of molecular biology in different circumstances of our daily life implies that it is very important that biomedical students deal with the principles and techniques underpinning its application. Human DNA fingerprinting is a major tool in identifying individuals and in evidence matching. However, this technique can be difficult to reproduce in practical classes.

Objectives

Create a practical class activity suitable for undergraduate students that elucidates how human oral microbiota is diverse and individually unique.

Methods

Here we report on distinct PCR profiles obtained when amplifying saliva DNA of a score of distinct individuals with RAPD primer BOXA1R. RAPD is a simple method efficiently used for discrimination between bacterial strains and is used in this instance to obtain personalized fingerprints of each individual's oral microbiota.

Conclusions

We present real results with undergraduate students confirming that this procedure is easily feasible in practical classes. Based on the results presented, we suggest a laboratory activity for undergraduate Molecular Biology / Microbiology students.

FEMS7-0754
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

GETTING INTO THE SWING OF THINGS - COMMITTING TO LIFELONG LEARNING AND DEVELOPMENT (FROM UNDERGRADUATES TO ACADEMICS)

B. Fahnert[1]
[1]*Cardiff University, Cardiff School of Biosciences, Cardiff, United Kingdom*

Backgrounds

In a rapidly changing world, where jobs are no longer for life or change beyond recognition, lifelong learning is required of everyone. Educators need to support learners early on in understanding this need and developing necessary skills and reflection. So initial Personal Development Planning prepares and becomes Continuous Professional Development. Equally, these educators need sufficient opportunity for pedagogic training, also perceived as valuable by undergraduates and benefitting academic career establishment/advancement. Teaching professionalism is not developed through traditional academic apprenticeship (i.e. doctoral studies), and -along with high graduate employability rates- has become a focus for Higher Education Institutions to boost institutional reputation.

Objectives

Better understand behaviour and drivers around committing to lifelong learning at two stages:

a) graduate attributes and career skills development
to inform practice when equipping/empowering undergraduates for successful transition into employment;
b) teaching training for early career academics
to achieve long-term benefit for all stakeholders.

Methods

Stakeholder and discourse analyses and ethically cleared mixed method studies (interviews, questionnaires) were conducted, qualitatively analysing experiences and perceptions of:

a) under/postgraduates, academics, employers, alumni;

b) teaching training participants and non-participating academics.

Conclusions

In both contexts behaviour is affected by conflicting demands on time at present and obtaining intangible benefits for an unknown future.

Undergraduates prioritise subject rather than career skills unless incentivised, which still does not guarantee meaningful engagement.

Challenges to teaching training were e.g. non-peer trainers, unfamiliarity with terminology and using qualitative methodology/findings.

Using identified drivers to tailor developmental provision and communication with stakeholders towards cultural change is outlined.

FEMS7-0678
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

SOCIAL NETWORKS AS KEY TOOLS IN MICROBIOLOGY DIVULGATION

J.D. Flores Felix[1], P. García-Fraile[2], P. Martínez-Hidalgo[1], M. Marcos-García[1], L. Celador-Lera[1], A. Díez-Méndez[1], X.A. Cruz-González[1], A. Jimenez-Gomez[1], R. Rivas[1]
[1]University of Salamanca, Microbiología y Genética, Salamanca, Spain
[2]Academy of Science of the Czech Republic, Department of Microbiology, Prague, Czech Republic

Backgrounds

YouTube is the major online video platform, with a high diversity of contents and over a billion of users from all around the world. Therefore, this platform has a high potential as education and divulgation tool. The combination of this platform with the social networks, such as Facebook or Twitter, has a great potential to introduce academic contents to a non-academic audience

Objectives

Our aim was to use of social networks as a tool to improve the diffusion of the Microbiology using multimedia contents guested in YouTube, increasing the number of potential spectators reached by our videos about microbiology techniques hosted in of YouTube channel.

Methods

We created different profiles in YouTube, Facebook and Twitter. The Youtube profile was linked to own channel, which has with 215 subscriptions and counts more than 30.000 video visualizations. On Facebook, we opened a Facebok Page named “Microbiología y Genética. Interacciones Planta-Microorganismo. USAL” (translated into English: Microbiology and Genetics. Plant-Microbes Interactions. USAL”) and on Twitter we created the profile @MicrobioUSAL. Then, we shared our Microbiology YouTube videos on our social networks.

Conclusions

We have seen than YouTube is a useful tool to disseminate microbiology contents into academic and non-academic fields and moreover, the use of social networks such as Facebook and Twitter to share the videos hosted on YouTube allowed us to increase the number of spectators, proved by peaks in the numbers of visualizations and minutes with clear activity during few hours following their shares on the social networks.

FEMS7-0501
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

"HOW ARE MICROBES USEFUL TO US?": MICROBIOLOGY LEARNING IN EUROPEAN RESEARCHER'S NIGHT.

A. Jiménez-Gómez[1], X. Cruz-González[1], J.D. Flores-Félix[1], L. Celador-Lera[1], A. Diéz-Mendez[1], E. Menéndez[2], M. Marcos-García[1], P. García-Fraile[3], R. Rivas[1]
[1]University of Salamanca, Department of Microbiology and Genetics, Salamanca, Spain
[2]Instituto de Ciências Agrárias e Ambientais Mediterrânicas, ICAAM, Evora, Portugal
[3]Academy of Science of Czech Republic., Institute of Microbiology, Prague, Czech Republic

Backgrounds

European Researcher's Night is one of the most important scientific events in Europe. It takes place every year on the last Friday in September. More than 30 countries and over 300 cities participate organizing scientific dissemination activities, showing to citizens what researchers really do for society and increasing the possibility to promote research careers to young people. In addition, Microbiology is one of the subjects which does not have an important relevance in the current high schools' curricula, and however has a big relevance in our lives. Moreover, microbiology requires from society the comprehension of many complicated concepts, which are easy to learn if taught in a practical way.

Objectives

In this context, our main objective was to offer society the possibility of seeing colonies of microorganisms cultured in Petri dishes, observing different samples under the microscope and showing the essential importance of microorganism in many key points of our daily life.

Methods

The activity was carried out in one of the microbiology laboratories of the University of Salamanca (Spain). The 40 participants were divided into small groups and were rotating to perform the different activities in one-hour sessions. The language used was suited to the different age of the participants (between 14 and 70 years old) and the topics presented were selected according to general society interests.

Conclusions

The experience was gratifying and positive not only for the participants but also for the researchers who organized the activity. It was an exchange of questions and answers which showed the general doubts of the society about Microbiology. The success of the activities shows us that the topics presented were interesting to both children and adults.

FEMS7-1711
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

STUDENT-GENERATED MULTIPLE-CHOICE PRE-EXAM QUESTIONS: AN EFFECTIVE TOOL FOR PARTICIPATORY LEARNING IN MICROBIOLOGY

M. Marvasi[1], M. Teplitski[2], M. Di Cesare[1], C.J. Krediet[3]
[1]Middlesex University, Natural Sciences, London, United Kingdom
[2]University of Florida, Water and Soil Science, Gainesville, USA
[3]Eckerd College, Marine Biology Department, St. Petersburg, USA

Backgrounds

It is commonly recognized that the act of creating questions enhances learners' understanding of course materials and promotes deep learning. Student-generated questions that involved higher cognitive skills (compared to a simple recall) have been linked to self-directed learning and improved conceptual understanding.

Objectives

In this multi-year study, students designed multiple-choice pre-exam questions during a Microbiology course aimed at higher levels of learning, followed by their discussion. Students were instructed to construct questions on the higher domains of Bloom's taxonomy. We tested the hypothesis that this intervention improves student learning.

Methods

Learning gains were measured as student achievement on the exam following the intervention, and compared to student achievement on the traditional exam (prior to which a review session was focused on instructor-led recitation of the key concepts). Over the duration of the experiment 162 students chose to participate in this study from 2009 to 2015.

Conclusions

Following the intervention in all years, average grade on the post-intervention exam increased by 7.4% points. It is important to point out that not all students benefited equally from this activity. The lowest quintiles improved their scores on the second exam in a range of 6.8% to 9.9% percentage points, respectively. Students who were in the 3rd quintile based on the results of the first exam demonstrated the highest achievement improving their performance on an average by 12.3% percentage points. The students within the forth quintile improved their performance by 10.8% percentage points. Such gains were not observed in the semesters when the intervention was not implemented.

FEMS7-1171
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

PARTICIPATORY WORKSHOPS: THERE IS NOT AGE TO DISCOVER MICROBIOLOGY

M. Orruño[1], *I. Arana*[2]
[1]*Faculty of Pharmacy - University of the Basque Country UPV/EHU, Immunology- Microbiology and Parasitology, Vitoria-Gasteiz, Spain*
[2]*Faculty of Science and Technology - University of the Basque Country UPV/EHU, Immunology- Microbiology and Parasitology, Leioa, Spain*

Backgrounds

Every year the University of the Basque Country (UPV/EHU) organizes the Science Week (ZientziaAstea). This annual event, aimed at audiences of all ages, tries to bring science to a non-specialist public by means of workshops, scientific shows, conferences and other activities. The microbiology has been present in this event from the first edition.

Objectives

To try to transmit microbiology in a more efficient and participatory way.

Methods

In recent years we have launched 2 workshops aimed at children and adults. The first workshop we implemented, *Microorganisms working*, tries to refute the bad image of microorganisms and to highlight their benefits and their ecological and industrial relevance. Among others, attendees can taste several foods and beverages obtained with microbial participation and discover that microorganisms are involved in the production processes of numerous common household product.

As the format was not the most suitable to keep the attention of the younger participants, we decided to launch a new workshop specifically aimed at children between 6 and 12 years, *Small microbiology for small scientists,* which tries to introduce children the microbial world through games and activities. They can use a microscope to discover the microbial diversity in natural samples, simulate the bacterial growth or become aware of their own *microbiological fingerprint*.

Conclusions

The workshops have been quite successful, with all the available places filled and a high level of satisfaction among attendees. In addition, the children's workshop has been exported to other fairs and adapted to schools.

FEMS7-0922
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

STANDARDISED METHODS IN PRACTICAL LAB WORK GENERATES IMPORTANT KNOWLEDGE FOR STUDENTS, SCIENTISTS AND THE FOOD SAFETY AGENCIES

C.S. Svanevik[1], B.T. Lunestad[1]
[1]National Institute of Nutrition and Seafood Research, Contaminants and Biohazards, Bergen, Norway

Backgrounds

The background for this work was to use standardised laboratory methods for both practical and theoretical teaching in seafood microbiology, where the achieved data also generates new knowledge for scientist and the government. As contaminated water is globally the main vehicle for microbial pathogens in most regions, we find that teaching future microbiologist and employees in the food industry on the importance of hygienically satisfactory water, microbiological analyses and how to ensure good water quality and safety, is highly relevant.

Objectives

This work present a complete experimental design for water analyses as a tool to teach students the methods and other key elements in microbiology, including food safety, environmental dissemination and survival of microorganisms, laboratory practices, water legislation and critical evaluation of results.

Methods

Analyses for the detection culturable bacteria (ISO 6222) and of fecal contamination in water (ISO 4788:1990, 4792:1990, 7899-2:2000) were used as an educational tool during a University course in seafood microbiology over a ten-year period (2006-2015). In addition to the lab results, the achieved knowledge among the students was examined by two retrospective questionnaires (about water analysis and lab course) conducted during June 2015. The questionnaires were circulated and made available for students back to 2006.

Conclusions

The questionnaires revealed that the laboratory course is highly appreciated, and that many students remembered important aspects of the water analysis, even after several years. The questionnaire results were consistent with our perception that some students find calculation of dilutions difficult to comprehend.

FEMS7-0976
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

HANDS-ON STAINING TECHNIQUES TO LEARN ABOUT BACTERIA

A. Martins[1,2], C. Dias[1,2], M.J. Fonseca[2,3], F. Tavares[1,2]
[1]Faculdade de Ciências da Universidade do Porto, Departamento de Biologia, Porto, Portugal
[2]CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos/InBIO Laboratório Associado, Microbial Diversity and Evolution, Porto, Portugal
[3]Universidade do Porto, Museu de História Natural e da Ciência da Universidade do Porto MHNC-UP, Porto, Portugal

Backgrounds

Practical work-based approaches have long been regarded as privileged educational tools in biology education. These practices lead to opportunities for students to develop conceptual, procedural and attitudinal skills.

Objectives

1. Assess the impact of a practical approach based on staining procedures on the learning process concerning bacteria;
2. Clarify misconceptions about bacteria;
3. Acquaint students with microscopy and staining techniques;
4. Foster the development of critical observational skills.

Methods

The activity, trialed and tested with 15/16 year-old students, included the observation and identification of prokaryotic cell structures using the optical microscope, and microscope slides prepared by the students and from collections. Data was gathered through a pre-/post-test design mixed-method approach with the use of an out-group. Statistical analyses were carried out using IBMS' SPSS v.21.

Conclusions

The findings revealed the overcoming of frequent bacteria-related misconceptions. Experimental group students listed notions such as "show non defined nucleus" [16 (post-test)vs.11(pre-test);$\chi2(1)=12.07;p<0.001$] or "with capsule which confers resistance" [6 vs. 0;$\chi2(1)=5.14$; $p=0.02$]. Both groups agreed that microscopy is important to diagnose bacteria (Expgroup:M=4.27;Dp=1.20;Z=340.5;p<0.001,d=0.14; Cntgroup:M=4.48;Dp=0.70;Z=300; p<0.001, d=0.01) and perceived laboratory work as an important learning tool (Expgroup:M=4.90; Dp=0.31;Z=465;p<0.001;d=0.42; Cntgroup:M=4.93; Dp=0.27;Z=378;p<0.001;d=0.50), allowing them to understand issues addressed in their classes (Expgroup:M=4.79;SD=0.48;Z=-1.42;p=0,15;d=0.16; Cntgroup:M=4.67;SD=0.48;Z=378;p<0.001;d=0.16). Regarding microscopy techniques, students emphasized their impact, namely at a motivational level, stating that these allow to "study bacteria in more detail".
The laboratory activity implemented had a positive effect in the participants' conceptual learning (morphology and physiology of the bacterial cell) and allowed the development of their procedural capabilities, as well as of their critical thinking skills.

FEMS7-0494
Communicating Microbial Science to Society: from Virtual to Hands-on Strategies

DEVELOPING COMMON CURRICULUM AND IMPROVING TEACHING APPROACHES OF MICROBIOLOGY EDUCATION

A. Netrusov[1], A. Trchounian[2]
[1]M.V. Lomonosov Moscow State University, Microbiology, Moscow, Russia
[2]Yerevan State University, Biochemistry- Microbiology & Biotechnology, Yerevan, Armenia

Backgrounds

Microbiology is one of the major subjects in Biology Education. New achievements of biology and formed basic and applied microbiology problems, on the one hand, the mobility of students and the labor market demands in microbiology specialists, on the other hand, require the development of a common (European or international) curriculum of academic microbiology [1].

Objectives

Microbiology in undergraduate education appears in two directions (areas) - General Microbiology and Applied (Medical) Microbiology.

Methods

By study and comparison of the microbiology programs for different universities, the following is proposed.

Conclusions

The new curriculum of General Microbiology may include three modules: (1) structure of the microbial cell (morphology), physiology, biochemistry and genetics of microorganisms; (2) biodiversity, ecology and systematics of microorganisms; (3) types, methods and directions of microbial biotechnology. A certain part of the program (to a quarter of volume) can include regional microbiology problems associated with microbial diversity, prevention of microbial diseases and environmental problems. It is important to highlight the objectives and output to ensure practical relevance. Special courses in undergraduate and graduate microbiology education in-depth subjects are in two areas and three modules.

To improve teaching approaches the problematic (interactive) lectures, practical classes and laboratory work, tests, and various forms of individual work should be implemented. The ratio of lectures and laboratory work should be offered approximately equal. Laboratory work must be carried out accordingly by general (international) protocols. Also important are consistent actions for the implementation to include assessment of the knowledge and learning feedback.

[1] Recommended curriculum guidelines for undergraduate microbiology education. ASM, 2012.

FEMS7-0270
EAM Workshop: Survival and Persistance

POST-TRANSCRIPTIONAL REGULATORY NETWORKS IN LEGIONELLA PNEUMOPHILA: KEY TO THE SURVIVAL IN EXTRA- AND INTRACELLULAR ENVIRONMENTS

C. Buchrieser[1], T. Sahr[1], G. Oliva[1], F. Impens[2]
[1]Institute Pasteur, Genomes and Genetics, Paris, France
[2]Ghent University, VIB Medical Biotechnology Center, Ghent, Belgium

Backgrounds

Legionella pneumophila, the causative agent of the pneumonia-like Legionnaires' disease, is commonly found in aquatic habitats where it multiplies within protozoa. *L. pneumophila* is able to survive and replicate in the hostile intracellular environment of a protozoan cell (or a human macrophage during disease). Once *L. pneumophila* has replicated to high numbers, it is released in the water (or the lung) where low nutrient extracellular conditions are present. To tolerate or exploit these conditions this bacterium has evolved a biphasic lifecycle wherein it alternates between a replicative and a transmissive phase. Genes that allow replication and to scavenge nutrients from the host are expressed when nutrients are available whereas genes that allow transmission and survival in the extracellular environment like virulence factors, motility and resistance against several stress factors are expressed, when nutrients are limited. The key regulator governing this adaptation thereby allowing the bacterium's intra- and extracellular survival is the RNA-binding protein CsrA.

Objectives

Our aim was to identify all targets of CsrA to understand how *L. pneumophila* adapts to the different conditions it encounters.

Methods

We used transcriptomics, proteomics, RNA-Immunoprecipitation followed by deep sequencing (RIPseq), together with biochemical, phenotypical and molecular analyses to identify the *L. pneumophila* CsrA targets genome wide.

Conclusions

Comparison of a wild type and a *csrA* mutant strain identified 478 RNAs with potential CsrA interaction sites and also led to the discovery of a new mode of action of CsrA that allows to regulate genes comprised in the same operon, independently.

FEMS7-0203
EAM Workshop: Survival and Persistance

BACTERIAL SURVIVAL STRATEGIES

M. Vicente[1], *A. Sánchez-Gorostiaga*[1]
[1]*Centro Nacional de Biotecnología- CSIC, Microbial Biotechnology, Madrid, Spain*

The role of FtsZ in bacterial survival

Filamentation helps bacteria to be less susceptible to antibiotics, to evade phagocytosis by the immune system, or to escape protist predators in marine environments (reviewed in Justice *et al.*, 2008 *Nat Rev Microbiol***6**: 162-168). Division-inhibited filaments provide a survival strategy against damages induced by host intracellular stress, nutrient limitation, inhibition of metabolic pathways or DNA damage produced by oxidative free radicals. Inhibition of the FtsZ activity by SulA is one of the filament-inducing mechanisms to counteract stress during host pathogen interaction. This process is reversible and once the activity of FtsZ is resumed at later infection steps the host-induced filaments can proliferate rapidly.

We found that the very low levels of FtsZ present in synthetically FtsZ-deprived cells (VIP205), has unexpected and severe pleiotropic effects on the global physiology of *E. coli* culminating in a reduced resilience that compromises bacterial survival. At the non-permissive conditions, cells of a FtsZ conditional mutant (PAT84) in which the GTPase activity and bundling are reduced, do not divide. In contrast to FtsZ-deprived VIP205 cells, the viability of the conditional PAT84 mutant or the SulA inhibited filaments is not affected because the amount of the FtsZ protein remains unperturbed and these filaments sustain no other damage that might compromise their survival.

Based on our results, we propose that the quest for new antibacterial compounds targeting FtsZ should be directed to decrease the number of FtsZ molecules instead of inhibiting the activity of the protein.

FEMS7-1328
FEMS-ESCMID

BACTERIOPHAGES AND ANTIBIOTIC RESISTANCE IN CYSTIC FIBROSIS

M. Brown-Jaque[1], L. Rodriguez Oyarzun[1], T. Cornejo-Sánchez[2], S. Gartner[3], J. de Gracia[3], S. Rovira[3], A. Alvarez[3], J. Jofre[1], J.J. González-López[2], M. Muniesa[1]
[1]Universidad de Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain
[2]Hospital Vall d'Hebron- Vall d'Hebron Institut de Recerca VHIR, Clinical Microbiology, Barcelona, Spain
[3]Hospital Universitario Vall d'Hebron, Cystic Fibrosis Unit, Barcelona, Spain

Backgrounds

Cystic fibrosis (CF) is a relevant genetic disease whose morbidity and mortality is related to lung affectation and its complications. Antibiotics are used to prevent bacterial colonization of patients; however, their effectiveness is compromised by the development of resistances. Bacteriophages are the least explored mechanism of transfer of resistance to antibiotics in clinical settings.

Objectives

To study the occurrence of bacteriophages harbouring seven antibiotic resistance genes (ARGs) in sputum samples of patients with CF to determine their role as vehicles of ARGs.

Methods

The study was conducted with sputum samples of 71 CF patients of ages from 9-63 years-old visiting the CF unit of Hospital Vall d'Hebron in Barcelona. A control group of 21 non-CF patients was included in the study. ARGs in phage packaged DNA were determined and quantified by TaqMan qPCR assays after phage DNA extraction.

Conclusions

bla$_{TEM}$ was the ARG with more positive samples and number of gene copies per ml of sputum (25%; 2.74 GC/ml), followed by *bla*$_{CTX\text{-}M\text{-}9}$ (27%; 2.46 GC/ml), *bla*$_{CTX\text{-}M\text{-}1}$ (25%; 2.30 GC/ml), *bla*$_{OXA\text{-}48}$ (16.7%; 2.03 GC/ml), and *qnrS* (8.3%; 1.19 GC/ml); *qnrA* and *mecA* showed the lowest values and prevalence. Prevalence presented statistical differences in patients < 18 years-old for *bla*$_{CTX\text{-}M\text{-}1}$ and *bla*$_{OXA\text{-}48}$. Generally ARGs presented greater prevalence in control group excepting *bla*$_{CTX\text{-}M\text{-}1}$ and *mecA* whose prevalence, even if low, is higher in the CF patients. Average quantity of ARGs of CF patients was higher than in the control group excluding *bla*$_{TEM}$ and *qnrA*.

FEMS7-0177
FEMS-ESCMID

LOW COST, HIGH THROUGHPUT, IN VIVO SCREENING OF NOVEL ANTIMICROBIALS FOR EFFICACY AND TOXICITY USING TRULARV™ GALLERIA MELLONELLA

O. Champion[1]
[1]*University of Exeter, BioSystems Technology, Exeter, United Kingdom*

Backgrounds

Multi-drug resistant (MDR) bacteria are an emerging threat in the modern world and new antimicrobial drugs are urgently being sought. One approach is to identify naturally occurring compounds produced by microorganisms. Historically, Actinobacteria have been a rich source of antimicrobials, but there are many species yet to be tested.

Objectives

In this study several thousand crude actinomycete extracts, were screened for antimicrobial activity, and approximately fifty lead extracts identified. The challenge we faced was how to further screen these extracts for efficacy and toxicity in an infection model. *Galleria mellonella* larvae can be used as an inexpensive, ethical and simple to use whole organism infection model. Previously reported studies have shown that there is good correlation between the results of *G. mellonella* infection experiments and existing drug efficacy data in humans.

Methods

Extracts were tested for efficacy and toxicity in, research grade *G. mellonella* (TruLarv™) larvae in a high-throughput assay. In this study larvae were infected with *Pseudomonas aeruginosa* NCTC10662 and concurrently dosed with one of the fifty crude extracts. To assess their efficacy, survival of the larvae was measured in a time course experiment. In parallel, to evaluate the toxicity of complex bacterial extracts maximum half lethal dose (LD_{50}) of the compounds in larvae was calculated.

Conclusions

We were therefore able to identify extracts containing bioactive compounds with low toxicity *in vivo* prior to their subsequent purification and testing in mammalian models.

FEMS7-1201
FEMS-ESCMID

ANTIBIOTIC RESISTANCE GENES IN THE BACTERIOPHAGES DNA FRACTION OF HUMAN FECAL SAMPLES AND BACTERIA ISOLATED FROM FECES

M. Brown-Jaque[1], W. Calero-Cáceres[1], P. Espinal[2], J. Rodríguez[2], E. Miró[2], J.J. González-López[3], T. Cornejo-Sánchez[3], J.C. Hurtado[4], F. Navarro[2], M. Muniesa[1]
[1]Universidad de Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain
[2]Hospital de la Santa Creu i Sant Pau, Microbiology service, Barcelona, Spain
[3]Hospital Vall d'Hebron- Vall d'Hebron Institut de Recerca VHIR, Clinical Microbiology, Barcelona, Spain
[4]Hospital Clínic, Microbiology service, Barcelona, Spain

Backgrounds

Bacteriophages are the most abundant entities in the world. They transfer genes between various bacterial genera and species acting as mobile genetic elements, contributing to bacterial genetic variability. Recent studies report that phage particles are reservoirs of antibiotic resistance genes (ARG) in different biomes suggesting the possible spread of ARG in phage particles by the human and animal population.

Objectives

To quantify ARGs in the viral DNA fraction of human fecal samples and to detect temperate bacteriophages carrying ARG incorporated as prophages in the genome of fecal bacterial isolates from healthy human carriers.

Methods

The study was performed using 139 human fecal samples from healthy individuals and 82 *Escherichia coli* and *Klebsiella pneumoniae* isolates from these fecal samples. The occurrence of inducible prophages in the strains was analyzed by mitomycin C treatment. ARGs were quantified by TaqMan qPCR assays in phage DNA extracted from feces and from phage particles induced from isolates.

Conclusions

bla$_{\text{TEMt}}$ was the most prevalent and abundant ARG in the viral DNA fraction of feces, followed by *qnrA*, *sul1*, *bla*$_{\text{CTX-M-9t}}$ and *armA*. Less than 10% of samples showed *qnrS*, *mecA*, *bla*$_{\text{CTX-M-1t}}$, and *bla*$_{\text{OXA-48t}}$.

bla$_{\text{TEMt}}$ and *bla*$_{\text{CTX-M-9t}}$ were the most prevalent ARG in phage particles induced from the strains. *sul1, bla*$_{\text{CTX-M-1t,}}$ *qnrS* and *qnrA* were less prevalent but also present in phages induced from strains.

Accordingly, ARGs are present in DNA of phage particles isolated from feces and in phages induced from fecal clinical isolates.

FEMS7-2914
FEMS-ESCMID

ANTIMICROBIAL PEPTIDES AND THE EVOLUTION OF RESISTANCE

B. Papp[1]
[1]*Biological Research Centre, Synthetic and Systems Biology Unit, Szeged, Hungary*

Cationic antimicrobial peptides (CAPs) are promising novel alternatives to traditional antibacterial agents, but the overlap in resistance mechanisms between small-molecule antibiotics and CAPs are unknown. Does evolution of antibiotic resistance decrease (cross-resistance) or increase (collateral sensitivity) susceptibility to CAPs? We systematically addressed this issue by studying the susceptibilities of a comprehensive set of antibiotic resistant *Escherichia coli* strains towards 24 antimicrobial peptides. Strikingly, antibiotic resistant bacteria frequently showed collateral sensitivity to CAPs, while cross-resistance was relatively rare. We identified clinically relevant multidrug resistance mutations that simultaneously elevate susceptibility to certain CAPs. Transcriptome analysis revealed that such mutations frequently alter the lipopolysaccharide composition of the outer cell membrane and thereby increase the killing efficiency of membrane-interacting antimicrobial peptides. On the basis of these findings, we identified CAP-antibiotic combinations that rescue the activity of existing antibiotics and selectively eradicate antibiotic resistant bacteria. Our work provides a proof of principle for the development of peptide based antibiotic adjuvants that enhance antibiotic action and block evolution of resistance.

FEMS7-1065
FEMS-ESCMID

A LONG-TERM EPIGENETIC MEMORY SWITCH CONTROLS BACTERIAL VIRULENCE BIMODALITY

I. Ronin[1], N. Katsowich[2], I. Rosenshine[2], N. Q. Balaban[1]
[1]The Hebrew University in Jerusalem, Racah Institute of Physics, Jerusalem, Israel
[2]The Hebrew University in Jerusalem, Department of Microbiology and Molecular Genetics, Jerusalem, Israel

Backgrounds

Phenotypic diversity in clonal populations of pathogens has been shown to enhance infection efficiency. When pathogens enter the host, sensing of environmental cues activate the expression of virulence genes. In contrast, the transition of pathogens from activating to non-activating conditions is poorly understood.

Objectives

The aim of this study was to examine whether phenotypic variability plays a role in the virulence of a model organism, enteropathogenic *E. coli* (EPEC), a human specific pathogen, during infection and in the transition to non-activating conditions.

Methods

We employed the high-throughput ScanLag methodology to systematically detect phenotypic variability in cultures of EPEC. The analysis revealed a bimodal growth rate in EPEC populations. Mathematical modeling followed by experimental validation exposed a mechanism leading to the establishment of a long-term hysteretic memory-switch resulting in the stable co-existence of non-virulent and hyper-virulent subpopulations.

Conclusions

We showed that long-term hysteretic memory drives the constitutive expression of the major virulence factors of the pathogen, even upon shifting to conditions that do not favor their expression, and identified *per* operon as the key factor for the hysteretic switch. This unique hysteretic memory-switch may be common in pathogenic bacteria, resulting in increased disease severity, higher infection persistency and improved host-to-host spreading.

FEMS7-0991
Food microbiology-safety

SHINING LIGHT ON BACTERIAL SPORE GERMINATION AND OUTGROWTH; PRESERVATIVE STRESS SURVIVAL OF BACILLI

S. Brul[1], J. Wen[2], L. Zheng[3], B. Swarge[3], Z. Tu[3], R. de Boer[1], J.P.P.M. Smelt[1], L.J. de Koning[4], E.M.M. Manders[5], C.G. de Koster[4]
[1]SILS-UvA, MBMFS, Amsterdam, The Netherlands
[2]SILS-UvA, MBMFS & Van Leeuwenhoek Centre for Advanced Microscopy, Amsterdam, The Netherlands
[3]SILS-UvA, Mass Spectrometry of Biomacromolecules & MBMFS, Amsterdam, The Netherlands
[4]SILS-UvA, Mass Spectrometry of Biomacromolecules, Amsterdam, The Netherlands
[5]SILS-UvA, Van Leeuwenhoek Centre for Advanced Microscopy, Amsterdam, The Netherlands

Bacterial spores are ubiquitous in nature and can withstand both chemical insults and physical stresses. Spores can survive food preservation processes and upon outgrowth cause food spoilage as well as safety risks. The risk is exacerbated by the heterogeneous germination and outgrowth behaviour of isogenic spore populations. Our studies focus on the mechanisms involved in normal spore structure development. Live-imaging systems, such as SporeTracker, allow us to analyse at single cell level spore germination and outgrowth. A major unknown factor in spore heterogeneity is the observation that not all cells sporulate simultaneously. Hence spore protein composition is likely inherently heterogeneous. Here we discuss approaches to synchronize *Bacillus subtilis* spore formation, and analyse spore proteins as well as germination and outgrowth physiology. Using in *B. subtilis* an IPTG-inducible *kinA* gene that allows us to titrate the amounts of phosphorylated KinA we reached, in agreement with previous analyses, a steep switch in the population from below 5% spores to above 95%. *B. subtilis* spores were heterogeneous in the intensity of GFP and mCherry labelled GerAA and GerD fusion proteins as well as their clustering into one or more foci (germinosomes). Upon triggering spore germination we followed Ger protein fluorescence dynamics as well as intracellular pH dynamics using IpHluorin under control of the ptsG promoter. Current efforts focus on Ger protein interaction with SpoVA channel proteins, coupling molecular data to heterogeneity in spore physiology, its description with mathematical models as well as the time-resolved analysis of the biogenesis of the protective spore coat.

FEMS7-0470
Food microbiology-safety

COMPARATIVE GENOMICS OF ST121 LISTERIA MONOCYTOGENES REVEALS CONSERVATION AND DIVERSITY OF PROPHAGES AND POTENTIAL FITNESS ADAPTATIONS TO FOOD AND FOOD PRODUCING ENVIRONMENTS

L. Ciolacu[1], K. Rychli[1], E. Harter[1], E. Wagner[1], A. Müller[1], A. Zaiser[1], M. Wagner[1], S. Schmitz-Esser[1,2]
[1]Institute of Milk Hygiene- Milk Technology and Food Science,
Department for Farm Animal and Public Health in Veterinary Medicine, Vienna, Austria
[2]Iowa State University, Department of Animal Science, Ames, USA

Backgrounds

The food-borne pathogen *Listeria (L.) monocytogenes* is able to survive for months and even years in food production environments. Particularly strains belonging to sequence type (ST)121 are often found to be abundant and to persist in the food production environments.

Objectives

The aim of the study was to elucidate genetic determinants characteristic for *L. monocytogenes* ST121.

Methods

We sequenced the genomes of 14 ST121 strains and compared them with currently available *L. monocytogenes* ST121 genomes. In total, we analyzed 70 ST121 genomes deriving from 16 different countries and sources.

Conclusions

All ST121 genomes show a high degree of conservation sharing at least 99.7% average nucleotide identity. The main differences between the strains were found in prophage content and prophage conservation. Additionally, we experimentally characterized two ST121 strain-specific genetic features: the transposon Tn*6188* and the novel stress survival islet (SSI-2) *lin0464*/*lin0465*. All strains harbor the novel SSI-2, consisting of homologues of the *L. innocua* genes *lin0464* and *lin0465,* a transcriptional regulator and a putative pfpI protease. Deletion of *lin0465* resulted in reduced survival under oxidative and alkaline stress conditions, suggesting a role in stress response. Tn*6188*, a transposon responsible for increased tolerance against quaternary ammonium compounds, present in 92.8% of all ST121 strains. 97.1 % of the ST121 strains contain a truncated *internalin A* (*inlA*) gene. Only one of the seven human ST121 isolates encodes a full-length *inlA* gene. *L. monocytogenes* ST121 strains are highly similar harboring highly conserved regions which most likely provide fitness adaptations to survival in food and food production environments.

FEMS7-1697
Food microbiology-safety

AN HIGH-THROUGHPUT MOLECULAR TOOL FOR HIGHLIGHTING THE EUKARYOTIC FOOD MICROBIAL COMMUNITIES: APPLICATION ON 12 FRENCH CHEESE VARIETIES

J. Denonfoux[1], S. Ferreira[1], L. Garnier[2], A.S. Sarthou[2], P. Bonnarme[2], F. Irlinger[2], E. Dugat-Bony[2]
[1]Genoscreen, Recherche- Développement et Innovation- Communautés microbiennes, Lille, France
[2]AgroParisTech- INRA, UMR Génie et Microbiologie des Procédés Alimentaires- GMPA, Thiverval-Grignon, France

Backgrounds

Cheese fermentation process is deeply related to culture and tradition. Indeed, the manufacturing processes shapes the chemistry and microbiology of cheeses, which contribute to express their organoleptic properties. France alone represents almost 1000 different cheese varieties harboring each a specific, dense microbiota (2–3 × 10^9 cells/g cheese). Recently, the use of metagenomics combined with high-throughput sequencing (HTS) technologies offer the opportunity to profile cheese microbial populations on a large scale. Even if the knowledge associated with the bacterial communities of cheeses is well documented, it remains still missing for the eukaryotic fraction.

Objectives

The GenoScreen company and the French National Institute for Agricultural Research (INRA) associated their expertise to develop a HTS tool for investigate the eukaryotic microbiota associated with 60 cheeses belonging to 12 traditional French cheese varieties.

Methods

The high throughput ITS2 amplicon sequencing associated with bioinformatic processing was used to characterize the fungal community composition down to the species level.

Conclusions

The results showed that major differences were observed between rind and core samples and also according to cheese varieties and manufacturing processes. Occurrence analysis revealed the presence of widespread taxa as well as operational taxonomic units (OTUs) specific to one or several cheese varieties. We highlighted as well some cheese varieties such as Saint-Nectaire and Soumaintrain still hosted unknown species that may account for a large proportion of the total community.
Finally, this molecular tool could be employed as a large-scale for deep microbial inventory in food processing-industry products.

FEMS7-0353
Food microbiology-safety

THE ORIGIN OF FOOD MICROBIOME: PROCESSING ENVIRONMENT AS SOURCE OF CONTAMINATION

D. Ercolini[1]
[1], *Italy*

Microorganisms inhabiting food-processing environments play an important role in defining the food initial contamination pattern and therefore influencing the shelf-life and the quality of the final product. Monitoring the presence of spoilage microorganisms in food-processing environment and mapping the possible contamination routes is necessary to prevent microbial spread along the processing chain and consequently the transition to the finished product. However, facility-resident microbiota was sometimes addressed as a source of microbes that may be beneficially involved in the manufacturing process.
Different food processing and manufacturing environments were analysed by culture-independent high-throughput sequencing, including beef, dairy and ready-to-eat meals processing plants. Swabs were collected from plant surfaces, tools and operators' hands. Moreover, food products from the same manufactures were also evaluated. The presence of a resident microbiota was highlighted, consisting of a few species well adapted to the considered environment, where food residues and exudates can act as substrates. This resident microbiota can be the source of food contamination and proliferate during storage to unacceptable levels, compromising food quality and safety. Nevertheless, depending on the type of manufacturing considered, food processing microbiota may sometimes play a positive role. Indeed, cheese manufacturing plants often harbour lactic acid bacteria, beneficially involved in the fermentative and ripening processes.
Therefore, depending on the nature of the micro-organisms and on the type of food manufacturing process, the environmental microbiota can exert positive functional activities or be a hazard for product quality and safety. Food-environment relationship deserves to be further explored, having the potential to affect the food processing dynamics and the quality of the final products.

FEMS7-1589
Food microbiology-safety

INTERACTIONS WITH DIFFERENT YEAST STRAINS PROMOTE A COMMON AND SPECIFIC TRANSCRIPTOMIC RESPONSE IN S. CEREVISIAE

J. Tronchoni[1], J.A. Curiel[1], P. Morales[1], R. Gonzalez[1]
[1]ICVV CSIC-Universidad de La Rioja-Gobierno de La Rioja, Enologia, Logroño, Spain

Backgrounds

New wine biotechnology trends include the use of non-*Saccharomyces* starter cultures. In a previous work, we showed the reciprocal effect of two wine yeast strains co-cultivated in scaled lab fermentation. Different non-*Saccharomyces* wine yeast species are potential strains to be used as co-inoculated starters.

Objectives

We analysed the transcriptional response to co-cultivation by an industrial yeast strain of *S. cerevisiae*. The *S. cerevisiae* wine strain was grown in a mixed culture with three different yeast strains, all of them can be found in a winery environment. Focused in the initial stages of wine fermentation, the answer of *S. cerevisiae* is analysed in order to uncover common and specific responses to different yeast interactions.

Methods

Fermentations were carried out in bioreactors using synthetic grape must to mimic industrial conditions. Experiments were done in triplicate. RNAseq data was used to analysis the yeast transcriptome.

Conclusions

Different yeast species promote a common response in *S. cerevisiae* with particular characteristics. The general answer found in the three yeast assayed is related to glucose uptake and glycolysis, membrane lipid metabolism, cell wall and the use of alternative nitrogen sources. One particularly interesting observation was that under conditions of co-cultivation with *T. delbrueckii*, *S. cerevisiae* by-passes normal nitrogen and glucose catabolite repression, up-regulating a series of genes that are usually transcribed under low concentrations of sugars (low affinity glucose transporters), or growing on non-preferred nitrogen sources. One illustrative example, found in all mixed cultures, is the *DAL* cluster gene family, up-regulated during co-cultivation.

Acknowledgments: MINECO(AGL2015-63629-R,RTC-2014-2186-2);EU(7FP-IRSES-GA612441);(JdC- JA.Curiel).

FEMS7-2605
Food microbiology-safety

DYNAMICS, ARCHITECTURE AND MATRIX CHARACTERIZATION OF BIOFILMS DEVELOPED BY CAMPYLOBACTER JEJUNI

H. Turonova[1], *R. Briandet*[2], *T.R. Neu*[3], *O. Tresse*[4]
[1]*University of Chemistry and Technology Prague, Dept. of Biochemistry and Microbiology, Prague 6, Czech Republic*
[2]*INRA, MICALIS, Paris, France*
[3]*Helmholtz Centre for Environmental Research – UFZ, Department of River Ecology, Magdeburg, Germany*
[4]*INRA, SECALIM, Nantes, France*

Backgrounds

Campylobacter jejuni has been reported as the leading cause of bacterial foodborne infections in developed countries, with significant increase over the past years. Despite the fastidious growth requirements, *C. jejuni* is able to survive in the environment without permanent loss of viability and virulence. The mechanisms responsible for its survival remain unknown, but one of the survival strategies might be linked to biofilm formation.

Objectives

This work focused on a detailed characterization of dynamics, spatial organization and matrix composition of *C. jejuni* biofilms, including the role of oxygen in the biofilm formation process.

Methods

The objectives were achieved by analysing biofilms of two characterized strains using confocal laser scanning microscopy, transmission electron microscopy (TEM), and fluorescent lectin binding analysis (FLBA) performed with 73 different lectins.

Conclusions

Both strains of *C. jejuni* were able to form biofilms within 17 h of cultivation. Biofilm architecture differed between the two strains, ranging from finger-like structure with voids and channels to compact multilayer-like structure that could be circumvented by a higher expression of *cosR* regulator. Exposure of cells to oxygen enriched conditions enhanced biofilm development. FLBA screening revealed strain-specific patterns with only 6 lectins interacting with the biofilm matrix of both strains. Interestingly, the biofilm matrix bound a fucose-specific lectin not previously detected within *C. jejuni*. Thioflavin T and curcumin assay paired with TEM highlighted the presence of amyloids in cell envelope without association with specific cell appendages. Taken together, these data provide new insights on structure and composition of *C. jejuni* biofilms.

FEMS7-1538
Future diagnostic approaches

FUNCTIONAL ANALYSIS OF THE GUT MICROBIOTA USING SINGLE CELL ISOTOPE PROBING

D. Berry[1]
[1]*University of Vienna, Department of Microbiology and Ecosystem Science, Vienna, Austria*

Backgrounds

Microbial communities are essential for human nutrition and health. It remains challenging, however, to identify the activity and function of microbial cells under natural conditions.

Objectives

This talk will focus on how microbes can be studied at the single cell level using molecular methods combined with isotope probing and chemical imaging tools.

Methods

I will introduce a new method to determine the general activity of cells using heavy water (D2O) and Raman microspectroscopy and will give examples of how this can be used to study gut microbiota utilization of a range of compounds, including mucosal proteins, host-derived amino acids, and dietary and mucosal sugars, polysaccharides, and glycans.

Conclusions

Single-cell activity measurements can give novel insights into the function of microbes in complex communities. Future directions for these exciting new techniques will also be highlighted.

FEMS7-0566
Future diagnostic approaches

NONINVASIVE COLORIMETRIC DETECTION OF FOODBORNE PATHOGENS BASED ON THE TRAPPING OF VOLATILE METABOLITES

E. Perret[1], M. Vrignaud[1], Z. Buniazet[1], P.R. Marcoux[2], P.L. Joly[1], J. Hue[1], I. Texier-Nogues[1]
[1]CEA, Division micro-Technologies for Biology and Healthcare, 38054 Grenoble cedex 9, France
[2]CEA, Division micro-Technologies for Biology and Healthcare, Grenoble, France

Backgrounds

We bring new low-cost disposable sensors for a convenient monitoring of microbial VOC (volatile organic compounds). There is a growing interest for the monitoring of microbial VOC as they can be used for noninvasive detection and identification of microorganisms. But the actual protocols often rely on expensive techniques, such as GC-MS (gas chromatography-mass spectroscopy).

Objectives

The method we develop is based on trapping and sensing VOC, thanks to transparent porous glasses. These show a very large surface for gas adsorption and can change color when capturing target gas. The main application we study is the detection of foodborne pathogens.

Methods

The porous glasses we use as sensors are made via polycondensation. It yields transparent nanoporous glasses with properties depending on monomers. Two types of sensors were made, that change color upon gas trapping: i) sensors for the detection of sulfide compounds, ii) sensors for enzyme-generated nitrophenol. Sulfide sensors are initially pale yellow and turn red-orange when detecting target VOC. Nitrophenol sensors are colorless and get yellow when trapping target gas. As in standardized methods, 25g of food sample was mixed with 250g of buffered peptone water. The mixture was homogenized in a stomacher bag and then incubated at 37°C. A sensor stuck to the bag wall could trap VOC emitted by liquid culture medium.

Conclusions

Salmonella can be detected either through the detection of H2S, or the nitrophenol emitted by C8-esterase enzymatic activity. *S. aureus* can be detected with the nitrophenol generated by a-glucosidase activity, in less than 8h (starting from 100 cfu/mL).

FEMS7-2585
Future diagnostic approaches

CYTO-WATER: A NEW PLATFORM FOR LEGIONELLA AND E. COLI RAPID DETECTION AND QUANTIFICATION IN ENVIRONMENTAL WATERS

E. Soria-Soria[1], V. Catalán[2], M.A. Yáñez[2], M.Á. Fernández-Fuentes[2], V. Mellado[1], E. Hill[3], D. Trouchet[4], J.M. Pérez[5], R. Goetzen[6], S. González[7]
[1]LABAQUA- S.A., Microbiology, Alicante, Spain
[2]LABAQUA- S.A., R&D, Alicante, Spain
[3]Memteq ventures Ltd., -, Stokesley, United Kingdom
[4]Bertin Technologies, -, Montigny-le-Bretonneux, France
[5]ICFO-The Institute of Photonic Sciences, -, Castelldefels - Barcelona, Spain
[6]microTEC Gesellschaft für Mikrotechnologie mbH, -, Duisburg, Germany
[7]CETAQUA, -, Cornellá de Llobregat - Barcelona, Spain

Backgrounds

Waterborne diseases are illnesses caused by pathogenic microorganisms that are present in contaminated water sources.

Analytical methods applied in diagnostic microbiology lab are usually performed manually and have some drawbacks, being one of them the long time-to results. For example *Legionella* detection by culture isolation can take up to 12 days.

New rapid methods could overcome the disadvantages of conventional analysis performed in the laboratory and achieve a rapid detection of waterborne pathogens to prevent outbreaks of waterborne diseases and the spread of waterborne pathogens.

Objectives

The main objective of CYTO-WATER project is to deploy a new platform for on-line monitoring of *Legionella* and *E. coli* in industrial and environmental water samples.

Methods

An automated water concentration module that includes Celltrap® concentrating filters, a microfluidic system for sample preparation and an image reader will be integrated resulting in the CYTO-WATER system.

Each component has been validated individually to make sure that they meet the market specifications. The integrated CYTO-WATER system will be validated according to ISO 16140-2:2016 in the laboratory comparing with conventional analysis methods (culture isolation and qPCR) in order to determine whether the method is suitable for detecting and quantifying microorganisms in water samples.

Conclusions

The CYTO-WATER platform could be placed, for example, in an industrial cooling tower, avoiding sampling and transport to the laboratory. The device will be a valuable tool for detection of possible bacterial contamination in a reduced timeframe with decision making capability.

FEMS7-1457
Future diagnostic approaches

ABSOLUTELY QUANTIFICATION AND CHARACTERIZATION OF VIABLE GUT MICROBIOTA

S. Tamburini[1], J.C. Clemente[2]
[1]Icahn School of Medicine at Mount Sinai, Department of Genetics and Genomic Sciences, New York, USA
[2]Icahn School of Medicine at Mount Sinai,
Department of Genetics and Genomic Sciences- Department of Medicine- Division of Clinical Immuno logy, New York, USA

Backgrounds

The human gut contains trillions of microorganisms that modulate health and disease in the host, but it is currently unclear how many total and viable bacteria live in a gram of fecal samples. Microbiome studies usually characterize the composition of the microbiota without taking into account the total amount of bacteria and their viability. These studies are thus limited by the fact that sequencing alone cannot distinguish whether the sequenced DNA originates from a viable, damaged, or dead bacterial cell.

Objectives

To absolutely quantify at single cell level the viable and dead population of bacteria that live in the human gut and the bacterial composition of these two bacteria subpopulation.

Methods

We applied fluorescence-activated cell sorting (FACS) combined with viability fluorescent markers, 16S ribosomal (rRNA) gene sequencing and statistical methods for absolutely quantifying at single cell level the concentration of total and viable bacterial cells and their taxonomic shape in healthy subjects

Conclusions

The FACS analysis identified that a gram of gut microbiota from healthy subjects contains on average $1.15x10^{11} \pm 0.3x10^{11}$ bacterial cells composed of a largest subpopulation of viable bacteria (72%) and a smallest one of dead bacteria (28%) with $8.46x10^{10} \pm 3.35x10^{10}$ and $2.65x10^{11} \pm 1.03x10^{10}$ cells/g, respectively. The 16S rRNA data showed consistent differences between viable and dead cells. Beta diversity analysis further highlighted the difference between the subset of viable and dead cells per subject and between subjects, suggesting that accounting for bacterial viability is critical in microbiome studies.

FEMS7-3300
Genomics, evolution, phylogeny

EXPLORING THE BOUNDARIES OF LIFE WITH GIANT VIRUSES

J.M. Claverie[1]
[1]*Mediterranean Institute of Microbiology, IGS UMR7256, Marseille, France*

Exploring the boundaries of Life with giant viruses

Beginning with the discovery of Mimivirus in 2003 [1], 15 years of exploration of this unexpected new branch of virology led us to the identification of four distinct families of large double-stranded DNA viruses all of which infect the same protozoan host, Acanthamoeba [2]. Beyond the immediate and simple fascination brought about by the mere size and shape of their particles, the alien nature and the diversity of their gene content is raising considerable doubt in our capacity to reconstruct their evolutionary history using the known genome modification processes within the accepted framework of Neo-Darwinism. Following a quick review of the known giant virus families and of their most distinctive properties, I will expose my doubts on the capacity of traditional phylogenetic thinking (and approaches) to ever figure out a scientifically sound theory for the origin of (all) viruses.

References

1] Raoult D, Audic S, Robert C, Abergel C, Renesto P, Ogata H, La Scola B, Suzan M, **Claverie JM** (2004). The 1.2-megabase genome sequence of Mimivirus. Science 306:1344-50. PMID: 15486256

2] Abergel C, Legendre M, **Claverie JM** (2015). The rapidly expanding universe of giant viruses: Mimivirus, Pandoravirus, Pithovirus and Mollivirus. FEMS Microbiol Rev. 39:779-96. PMID: 26391910.

FEMS7-2210
Genomics, evolution, phylogeny

MAPSEQ: BRINGING SPEED, ACCURACY AND CONSISTENCY IN RIBOSOMAL RNA SEQUENCE ANALYSIS

J.F. Matias Rodrigues[1], J. Tackmann[1], T.S.B. Schmidt[2], C. von Mering[1]
[1]University of Zurich, Institute of Molecular Life Sciences, Zurich, Switzerland
[2]European Molecular Biology Laboratory, Bioinformatics Group, Heidelberg, Germany

Backgrounds

Metagenomic sequencing has become crucial to the study of microbial communities, but meaningful analysis and integration of such data has been hampered by technical limitations, between-study variability and inconsistencies between taxonomies used.

Objectives

With the improvements in speed, accuracy and consistency of rRNA marker gene analysis by MAPseq we aim to enable a more consistent comparison of results and conclusions between metagenomic studies so that the rich context provided by existing data can be more readily explored.

Methods

The increased computational efficiency and higher accuracy are due to several innovations in the MAPseq algorithm tailoring it to sequence searches in large 16S/18S rRNA databases consisting of highly similar sequences. These innovations include an improved kmer-counting method based on a pre-clustered database, full alignment for high scoring segment pairs, and a sensitive algorithm to compute classification confidence. In addition, we provide a curated reference of full-length rRNA sequences pre-clustered into hierarchical OTUs and with several taxonomic categories which include, among others, the Living Tree Project and NCBI taxonomies.

Conclusions

MAPseq is a framework for reference-based rRNA metagenomic analysis that is 15x faster and up to 30% more accurate than existing solutions, providing in a single run multiple taxonomy classifications and hierarchical OTU mappings, for datasets of virtually any size. When inferring OTU presence and abundance, MAPseq achieves superior consistency over uclust when analyzing sequencing runs targeting different regions (V1V3 and V3V5) of the 16S rRNA gene in identical samples from the Human Microbiome Project.

FEMS7-0340
Genomics, evolution, phylogeny

CONFECTION - A CONJUGATIVE PLASMID MEDIATED METHOD TO INTRODUCE CRISPR-CAS9 GENOME EDITING COMPONENTS INTO EUKARYOTIC CELLS

P. Ruotsalainen[1], *M. Jalasvuori*[1]
[1]*University of Jyväskylä, Department of Biological and Environmental Science, Jyväskylä, Finland*

Backgrounds

The RNA-directed genome editing via CRISPR/Cas9 is an efficient and highly specific tool for gene editing of eukaryotes. At present CRISPR/Cas9 gene engineering system is usually delivered to eukaryotic cells by a viral vector. However the drawbacks of viral vectors are their immunogenicity and cytotoxicity and potential insertional mutagenesis (integration of viral genome into eukaryotic genome). Also the packaging capacity is low and the preparation of viral vector is laborious and requires appropriate bio-safety facilities.

Objectives

The aim of our research is to develop a novel transfection method named confection.

Methods

In confection plasmids expressing CRISPR/Cas9 gene engineering system, are transferred to eukaryotic cells via bacterial conjugation channels provided by conjugative IncP plasmid. These plasmids have been shown to be able to deliver DNA from bacterial cells to eukaryotic ones.

Conclusions

Compared to viral vectors, larger gene sequences can be transferred at once by confection. Bacterial cells also provide a self-renewing and replicating editing library, which is easy to modify for targeting desired genes. Confection may have various *in vitro* and *in situ* genome editing applications.

FEMS7-2352
Genomics, evolution, phylogeny

WHOLE-GENOME SEQUENCING IMPROVES MS-BASED PROTEOTYPING OF CLINICALLY-RELEVANT BACTERIA

F. Salvà-Serra[1], H. E. Jakobsson[1], L. Gonzales-Siles[1], R. Karlsson[1], F. Boulund[2], D. Jaén-Luchoro[1], S. Yazdan[1], A. Johnning[3], E. Kristiansson[3], E. R. B. Moore[4]
[1]University of Gothenburg, Department of Infectious Diseases, Gothenburg, Sweden
[2]Karolinska Institute, Department of Microbiology Tumor and Cell Biology, Stockholm, Sweden
[3]Chalmers University of Technology, Department of Mathematical Sciences, Gothenburg, Sweden
[4]University of Gothenburg, Culture Collection University of Gothenburg, Gothenburg, Sweden

Backgrounds

The prevalence of bacteria with resistance to multiple antibiotics is increasing worldwide. The primary reason can be attributed to the global misuse of antibiotics. To diminish this problem, it is critical to be able to detect and characterise bacteria causing infection rapidly and correctly. Mass-Spectrometry-based 'Proteotyping' is a rapid, culture-independent method that can detect and identify bacteria and their metabolic features (e.g., antibiotic resistance and virulence factors). However, Proteotyping relies on a comprehensive and reliable genome sequence database.

Objectives

1- Recover whole-genome sequences from public databases.

2- Determine whole-genome sequences of additional strains of clinically-relevant and closely-related bacterial species.

3- Verify the taxonomic assignment of the whole-genome sequence data.

4- Incorporate new sequence data into an internal database to increase the accuracy and resolution of Proteotyping results.

Methods

Bacteria are disrupted with bead beating; cellular proteins are trypsin-digested into peptides. These peptides are analysed, using LC-MS/MS, to obtain mass spectra profiles that are matched against a reference peptide database. The determined peptide sequences are matched against genome sequence database. The database includes sequences of all complete genomes from RefSeq, additional genomes from GenBank and additional *in-house* sequenced genomes. The taxonomic affiliations of the genome sequences are verified, using ANIb.

Conclusions

More than 100 whole-genome sequences have been determined. Enlarging and curating the genome sequence database has markedly improved the accuracy and resolution of proteomic analyses for Proteotyping. Misclassified genomes exert direct and marked effects, dramatically decreasing the number of discriminatory peptides and reducing the efficacy of Proteotyping.

FEMS7-0017
Genomics, evolution, phylogeny

INFECTION OF MICROBIOTA BY BACTERIOPHAGES CAN BE CONSIDERED A NEW GROUP OF VIRAL DISEASES OF MAMMALS INCLUDING HUMANS

G. Tetz[1], V. Tetz[1]
[1]Human Microbiology Institute, Human Microbiology, New York, USA

Backgrounds

Increased intestinal permeability and translocation of gut microbiota from the intestinal lumen predispose patients to—and may be one of the main triggers of various mammalian diseases.

Objectives

The objective of this study was to assess the effect of microbiota treatment with bacteriophages on the intestinal permeability *in vivo* and to evaluate the possibility that the infection of microbiota by bacteriophages may affect mammals.

Methods

We studied alterations in the host macroorganism and increased intestinal permeability as a result of a direct effect of bacteriophage cocktail. Healthy adult, albino Wistar rats, weighing 180–220g were given daily (for 10 days) phage cocktail (1.5 ml of $1x10^6$ plaque forming units/ml) active against Enterobacteriaceae, Staphylococcaceae, Streptococcaceae, and Pseudomonadaceae families. The lactulose-mannitol ratio was used as a marker of intestinal permeability. Circulating immune complexes (CIC) were evaluated as markers of endogenous intoxication. Metagenomic analysis was used to characterize the composition of microbiota before and after phage challenge.

Conclusions

Results: After 10 days of challenge, the rats showed weight loss, decreased activity. They displayed a significantly elevated lactulose:mannitol ratio with the mean increase 2.4 fold. The level of CIC was more than 2.5 times higher as compared to before treatment, indicating endogenous intoxication, caused by leaky gut. Metagenomic analysis revealed phage-induced altered microbiota composition.

Conclusions: To our knowledge, this study for the first time indicates the link between bacteriophages and mammalian pathologies associated with increased intestinal permeability such as systemic inflammatory and psychological or autoimmune disorders. This study demonstrates that increased intestinal permeability may be induced by bacteriophages that affect microbiota. We propose that infection of microbiota by bacteriophages can be considered a new group of viral diseases of mammals including humans.

FEMS7-2329
Genomics, evolution, phylogeny

INSIGHTS INTO THE EVOLUTION OF PATHOGENS THROUGH MUMMY STUDIES

A. Zink[1], *F. Maixner*[1]
[1]*Institute for Mummies and the Iceman, EURAC Research, Bolzano, Italy*

Insights into the evolution of pathogens through mummy studies

The molecular analysis of ancient pathogen DNA represents a unique opportunity for the study of infectious diseases in skeletal and mummified human remains. Within the last years, a wide range of bacterial, protozoal and viral infections have been detected in ancient tissue samples by the characterization of specific DNA fragments. The introduction of next generation sequencing (NGS) technologies in the study of ancient human remains has further improved the opportunity to study human evolution, population dynamics, and disease evolution. In several studies, new important findings regarding the evolution and spread of some major infectious diseases, such as plague, tuberculosis and leprosy were revealed.

One of the first mummies in which this technology has been successfully applied, is the Tyrolean Iceman, commonly known as Ötzi. By using metagenomic diagnostics and targeted genome capture, we determined the presence of the stomach pathogen *H. pylori* and reconstructed its complete genome. Subsequent sequence analysis classified the ancient *H. pylori* as a virulent strain that is now associated with inflammation of the gastric mucosa. Comparative analysis of ancient housekeeping gene fragments and comparative whole-genome analyses assigned the 5,300-year-old bacterium to a nearly pure representative of the bacterial population of Asian origin, suggesting that the African *H. pylori* population arrived in Europe within the past few thousand years, which is later than previously proposed.

In this presentation, the major results of the application of NGS for the study of pathogens are presented and discussed with regard to the perspectives and probable limitations in mummy research.

FEMS7-2919
Global metabolic cycles

METABOLIC PATHWAYS ASSOCIATED WITH SULFUR METABOLISM

I. Cardoso Pereira[1]
[1]*Instituto de Tecnologia Quimica e Biologica Antonio Xavier- Universidade NOVA de Lisboa, Bacterial Energy Metabolism Laboratory, Oeiras, Portugal*

Metabolic pathways associated with sulfur metabolism

Prokaryotes play a critical role in the biogeochemical sulfur cycle, both in the oxidation (and disproportionation) of reduced sulfur compounds, performed by chemotrophic and phototrophic sulfur oxidizing bacteria, as well as in the reduction of sulfate or other sulfur oxides performed by sulfate reducing bacteria and archaea. These microorganisms have a considerable impact on the geochemistry of anoxic environments as well as the global redox state of the planet over geological times.

The metabolic pathways involved in dissimilatory sulfur metabolism involve a series of proteins that are unique to these pathways and common to reducing and oxidizing organisms, indicating a specific role in sulfur chemistry and a common evolutionary origin [1].

In this talk I will discuss recent work on the role of some of these proteins in sulfur metabolism, with a special focus on the DsrAB/DsrC/DsrMKJOP system [2], the QmoABC/AprAB system and the Hdr system, and how they contribute to energy conservation.

[1] 12. Rabus R, Venceslau SS, Wöhlbrand L, Voordouw G, Wal JDl and Pereira IAC (2015) A Post-Genomic View of the Ecophysiology, Catabolism and Biotechnological Relevance of Sulphate-Reducing Prokaryotes. Adv. Microb. Physiol. 66, 55-321.

[2] Santos AA, Venceslau SS, Grein F, Leavitt WD, Dahl C, Johnston DT, Pereira IAC (2015) Science, 350, 1541

FEMS7-0713
Global metabolic cycles

UNVEILING MICROBIAL FUNCTIONAL CAPACITIES AND ACTIVITIES IN THE N CYCLE AT THE ANTARCTIC SUMMER COASTAL WATERS

B. Díez[1,2], M.E. Alcamán[1,2], F. Laura[2,3]
[1]Pontificia Universidad Católica de Chile, Molecular Genetics and Microbiology, Santiago, Chile
[2]Center for Climate and Resilience Research CR2, -, -, Chile
[3]Universidad de Concepción, Department of Oceanography, Concepción, Chile

Backgrounds

Antarctic marine waters are defined as one of the most HNLC regions of the planet. Nitrate (NO_3^-) and ammonium (NH_4^+) are the major forms of inorganic nitrogen used by phytoplankton. However, the significance of other N pathways in this cycle is poorly understood in Antarctic waters.

Objectives

The structure, functional activity and rates related to the N cycle of the microbial communities inhabiting surface waters of Chile Bay (62º 27’ 600’’ S 59º 40’ 600” W), in the South Shetland Islands, were analyzed during the summer of 2014.

Methods

Metagenomics, metatranscriptomics and *in situ* assimilation of ^{15}N, $^{15}NO_3$, and $^{15}NH_4$ as well as ^{13}C isotopes were the methodology used.

Conclusions

Our results under a typical HNLC condition, 1.9 µM P (PO_4) and 26.4 µM N (NO_3 and NO_2), showed low N_2 fixation rates (0.07 to 0.53 nmol N L^{-1} d^{-1}) and high $^{15}NO_3$ and $^{15}NH_4$ assimilations rates during light condition. The later processes are coupled with high ^{13}C fixation rates (600-2300 nmol C L-1 d-1), and a markedly high phototrophic activity during daytime by Calvin related to Diatoms and Haptophyta. Our analysis demonstrated the major abundance and activity of Proteobacteria (Gammaproteobacteria and Alphaproteobacteria), and Flavobacteria. Interestedly, ammonia-oxidizing archaea belonging to the genus *Nitrosopumilus* (Thaumarchaeota) and some Proteobacteria (*Nitrosomonas*) seem to be active chemoautotrophs, responsible for the nitrification process, according with the *amo*A gene activity. Thaumarchaeota are also possibly responsible for the nitrifier-denitrification (detoxifying nitrite), with potential contribution to N_2O production in these marine Antarctic waters, according with the *nir*K gene activity.

FEMS7-2312
Global metabolic cycles

SULFIDE AT LOW PH IS NOT YOUR ENEMY, BUT YOUR ALLY

A.P. Florentino[1], A.J.M. Stams[1], I. Sanchez Andrea[2]
[1]Wageningen University, Microbiology, WAGENINGEN, The Netherlands
[2]Autonomous University, Microbiology, Madrid, Spain

Backgrounds

Mining industry activity and the natural oxidation of metallic sulfide-ores generate acidic sulfur-rich and highly metalliferous waters, generally termed acid mine drainage. This is of great environmental concern as some heavy metals are highly toxic and produce long term harm to water quality and biodiversity.

Objectives

Prokaryotes of the reductive sulfur cycle play a role in the remediation of such waters, as the reductive process of sulfur compounds leads to the formation of sulfides that can precipitate metals as metal sulfides. Besides, the process consumes protons, which leads to an increase of pH to circumneutral values. Therefore, our objective was to isolate novel microbial key player of this process.

Methods

Enrichments of sulfate and sulfur reducers with various electron donors at low pH and mesophilic conditions were obtained from sediments of the acidic Tinto river (Spain).

Conclusions

This resulted in isolation of a whole set of novel acidophiles: sulfate reducing bacteria (two novel proposed species *Desulfosporosinus acididurans* and *Desulfosporosinus methalovorans,* and a new genus proposed as *Desulfobacillus acidiphilis*); sulfur reducers (a new genus, *Lucifera butyrica*, and a new species, *Desulfurella amilsii)*. Functional characterization of the isolates combined with proteomic analysis under specific conditions have helped to gain insights into their metabolism, unveiling novel pathways in the reductive sulfur cycle and novel strategies to cope with low pH. The ability of those microorganisms to thrive at acid rock drainage environments and to face moderate concentrations of heavy metals in solution make them potential candidates to combine ecological relevance with biotechnological application.

FEMS7-2920
Global metabolic cycles

METHANE CYCLING IN A LARGE FRESHWATER LAKE

B. Schink[1], *J. Deutzmann*[1]
[1]*University of Konstanz, Biology, Constance, Germany*

Methane is produced in the deeper layers of the sediment of Lake Constance and is reoxidized nearly completely within the oxygen-supplied upper 3 – 5 mm of the sediment. The methane-oxidizing community is rather heterogeneous, including largely type I methanotrophs. Novel methane-oxidizing bacteria were isolated in oxygen-limited cultivation devices, and growth could be improved by addition of methanol-oxidizing partners. At methane seeps in the Eastern part of the lake, higher numbers of methanotrophs were detected, and the predominant methane oxidizers differed also qualitatively from those at reference sites. Besides aerobic methane oxidation, nitrite-dependent anaerobic methane oxidation by NC10-like bacteria appeared to be dominant, especially in sediments at greater water depths (> 40 m). Sulfate-dependent methane oxidation was not detected. Our results show that the diversity of methane oxidation in a freshwater lake is far greater than expected until only a few years ago.

FEMS7-0515
Global metabolic cycles

SELECTIVE INHIBITORS OF MICROBIAL SULFATE REDUCTION: ASSESSING RESISTANCE TO AND RESILIENCE AGAINST INHIBITION

M. Stoeva[1], G. Nalula[2], N. Garcia[2], Y. Cheng[3], J. Coates[1]
[1]UC Berkeley, Plant and Microbial Biology, Berkeley, USA
[2]UC Berkeley, Integrative Biology, Berkeley, USA
[3]Lawrence Berkeley National Laboratory, Climate and Ecosystem Sciences Division, Berkeley, USA

Backgrounds

Hydrogen sulfide is a toxic and corrosive gas, produced by the activity of sulfate-reducing microorganisms (SRM). Owing to the environmental, economic and human-health consequences of sulfide, there is interest in developing inhibitors of SRM. Perchlorate is emerging as a promising inhibitor; however, a comprehensive analysis of its effects in communities is lacking.

Objectives

The aim of this work is to assess the inhibitory dynamics of perchlorate in sulfidogenic communities.

Methods

Sulfidogenic continuous-flow systems are treated with perchlorate and SRM number, sulfide production and community structure are monitored pre-, during and post-treatment. The data generated is used to create a mathematical model of how a simple SRM community responds to perchlorate.

Conclusions

Sulfide production decreases with perchlorate treatment, and 16S-amplicon sequencing and qPCR show a drop in SRM abundance; all parameters recover post treatment. Rates of inhibition and rebound fit the generated model, confirming that perchlorate acts as a bio-competitive inhibitor, slowing the growth of SRM. The model is subsequently validated by varying parameters identified as important: the perchlorate concentration and the level of surface-attachment. Further, we quantify susceptibility to perchlorate across several SRM in various contexts and are currently characterizing the mechanism of this susceptibility via proteomics. Finally, indirect effects of perchlorate (bio-competitive exclusion of SRM by dissimilatory perchlorate-reducing bacteria, DPRB) are tested by amending reactors with DPRB. Results confirm that at low perchlorate concentrations, SRM inhibition results from indirect effects.

This study thus provides a holistic overview of the sensitivity of sulfidogenic communities to perchlorate, as well as mechanisms underlying these patterns.

FEMS7-2887
Modeling microbial systems

THE MULTIPLEX PHASE INTERLOCKER – A NOVEL AND ROBUST MOLECULAR DESIGN SYNCHRONIZING TRANSCRIPTIONAL CELL CYCLE DYNAMICS

T.D.G.A. Mondeel[1], C. Linke[1], S. Tognetti[2], M. Loog[3], H.V. Westerhoff[1], F. Posas[2], M. Barberis[1]
[1]University of Amsterdam, Synthetic Systems Biology, Amsterdam, The Netherlands
[2]Universitat Pompeu Fabra, Departament de Ciències Experimentals i de la Salut, Barcelona, Spain
[3]University of Tartu, Institute of Technology, Tartu, Estonia

Backgrounds

The eukaryotic cell cycle is robustly designed, with networks of interacting molecules organized in regulatory motifs that ensure its precise timing. This is governed by a transcriptional oscillator interlocked with waves of cyclin-dependent kinase (cyclin/Cdk) activity. Although details about cyclin transcription are available, a lack of understanding exists about regulatory motifs responsible for the precise timing of waves of cyclin activation.

Objectives

We have recently identified a transcriptional mechanism that regulates the relative timing of waves of mitotic (Clb) cyclin expression in budding yeast. This involves the Forkhead transcription factor (TF) Fkh2. Here we investigate the robustness of molecular designs interlocking Clb waves through Fkh2-mediated signaling, with the aim to unravel the network motif(s) responsible for timely cyclin/Cdk1 dynamics

Methods

An integrated computational and experimental framework is presented. A kinetic model of the cyclin/Cdk1 network is simulated under a quasi-steady state assumption, and fitted to *in vivo* time course data of Clb dynamics. Robustness analyses are then performed by testing 1024 possible network motifs for their ability to fit Clb oscillations. Experimental validation supports computational analyses, revealing the Clb/Cdk1-Fkh2 axis to be pivotal for timely transcriptional dynamics.

Conclusions

A novel regulatory motif synchronizing Clb waves, coined as *Multiplex Phase Interlocker*, is unraveled. This motif uniquely describes a molecular timer (TF) that relies on separate inputs (cyclin/Cdk1 complexes) converging on a common target (TF itself). Within the motif, a progressive TF (Fkh2) activation may be realized by the sequential Clb/Cdk1 complexes. Altogether, our integrative approach reveals a conserved design principle in cell cycle control.

FEMS7-0124
Modeling microbial systems

THERMODYNAMIC ANALYSIS OF THE METABOLISM OF AMMONIA-OXIDISING BACTERIA SUGGESTS A NOVEL MECHANISM OF ENERGY HARVESTING

R. González-Cabaleiro[1], C. Tom[2], O. Dana[1]
[1]Newcastle University, School of Chemical Engineering and Advance Materials, Newcastle upon Tyne, United Kingdom
[2]Newcastle University, School of Civil Engineering and Geosciences, Newcastle upon Tyne, United Kingdom

Backgrounds

The biochemistry and bioenergetics of the metabolism of ammonia-oxidising bacteria (AOB) shows an inefficient and constrained metabolism. Only 52.8% of the energy released when a mole of ammonium is consumed and less than half of the electrons liberated in the process can be directed to the autotrophic anabolism. Paradoxically, AOB seem to thrive in challenging conditions: growing readily in virtually any aerobic environment, except the laboratory pure culture.

Objectives

We have sought to understand this enigma by analysing in detail the metabolism of the AOB.

Methods

We have used biochemical and thermodynamic modelling to calculate the maximum possible energy harvest of AOB metabolism. The results obtained have been compared with the experimental growth yields measured.

Conclusions

The model predicts a maximum autotrophic yield of 0.16 gBio/gN (0.13 gBio/gN when maintenance is considered). Observed yields should be lower than this value, but in reality they vary between 0.04 and 0.45 gBio/gN. Thermodynamics and experimental data can only be reconciled if AOB have an extra source of energy at least some of the time. Chemoheterotrophic growth of AOB only offers a partial explanation since it only occurs in specific conditions and there is ample evidence of CO_2 fixation by AOB. Alternative source of energy could be realised if the ammonia monooxygenase reaction was coupled to an energy harvesting mechanism. In particular, co-metabolism could confer a direct energy benefit on the cell. If this was true, it would explain the robust growth of AOB in mixed communities and account for the putative inefficiency of their metabolism.

FEMS7-1729
Modeling microbial systems

IMPROVED PROMOTER SEQUENCE MODELS FOR DE NOVO TRANSCRIPTION FACTOR BINDING SITE PREDICTION IN BACTERIA

I. Sultan[1], S. Schbath[1], V. Fromion[1], P. Nicolas[1]
[1]INRA-Institut National de la Recherche Agronomique,
Applied Mathematics and Computer Science from Genomes to the Environment MaIAGE,
Jouy en Josas, France

Backgrounds

Unravelling gene regulatory networks helps understanding many features about an organism. Over the past decades, powerful algorithms and approaches have been developed for the discovery of transcription factor binding sites (TFBSs) but these tools are still unable to automatically identify *de novo* the main regulons of a bacteria from genomic and transcriptomic data.

Objectives

In this work, we present a new statistical model and Markov Chain Monte Carlo (MCMC) algorithm that attempt to overcome some limitations of the available approaches. In particular, available algorithms for motif discovery based on mixture models do not take into account the overlapping of TFBSs. Whereas, available data on *Escherichia coli* suggest that overlapping is a major feature of bacterial promoter architecture (more than one third of the binding sites overlap with at least one other site). Moreover, with the advent of precise transcription start site (TSS) maps derived from RNA-Seq protocols targeting transcript 5'-ends, it is now relevant to examine the exact position in the promoter region when searching for TFBSs.

Methods

Our statistical model of promoter sequences accounts for the overlapping between TFBSs as well as their exact positioning with respect to the TSS. All the parameters are estimated in a Bayesian framework using a dedicated MCMC algorithm. The algorithm is trans-dimensional to allow adjusting the width of position weight matrix describing each motif and the number of parameters to describe its preferred position.

Conclusions

Results on *Listeria monocytogenes* regulatory network will be presented and strategies to incorporate expression data across conditions will be discussed.

FEMS7-1941
Modeling microbial systems

CHARACTERISATION OF METABOLIC BURDEN IN BACTERIAL SYSTEMS

S. Wagner[1], K. Pflüger-Grau[1], A. Kremling[1]
[1]Technical University of Munich, Systems Biotechnology, Garching, Germany

Backgrounds

In biotechnological approaches it is often necessary to introduce a multiplicity of genes into a bacterial host system as e.g. *Escherichia coli*. Such a heterologous expression can cause a competition for limited cellular resources as ribosomes, polymerases, and other precursors.

Objectives

In order to understand the cellular processes and limitations during foreign protein production, we set out to quantitatively describe the distribution of the resources between the intrinsic processes needed for maintenance and the extra load introduced by the heterologous pathway.

Methods

To this end, we analysed cellular behavior under a systematic variation of heterologous load. Additionally to the process parameters, we are able to record *on-line* the transcriptional and translational rates of the heterologous load. This is achieved by the introduction of a specially-designed plasmid into the cells that allows us to monitor and quantify the expression of a desired gene *in vivo*. To assess the cellular fitness, we investigated the cellular capacity as described by Ceroni *et al***.** (Quantifying cellular capacity identifies gene expression designs with reduced burden. Nature Methods 12: 415–418**,** (2015)) in terms of resource-distribution.

Conclusions

With this setup, we were able to quantify mRNA and protein production rates, as well as stabilities. Besides the expression process, cellular fitness was monitored. Because of the easily measurable fluorescence signals, this system has the potential to be a valuable tool for the systematic high-throughput analysis of pathway fine-tuning. Bottlenecks can be identified and, at the end of the day, circumvented or avoided.

FEMS7-0007
One health initiative

EVALUATION AND ASSESMENT OF ANTIBIOTIC RESISTANCE IN THE PRISTINE ESTUARY OF INDIAN SUNDARBANS, A WORLD HERITAGE SITE

A. Bhattacharyya[1], *M. Bhattacharyya*[1], *A. Ghosh*[2]
[1]*University of Calcutta, Biochemistry, Kolkata, India*
[2]*Bose Institute- Kolkata, Biochemistry, Kolkata, India*

Backgrounds

Antibiotic resistance is one of the principle challenges throughout the globe for the clinical microbiologists in recent times. Previously, only the hospital wastes or highly contaminated sewage in densely populated areas were found to be the point sources of antibiotic resistant bacteria but very recently the so called pristine natural environments and even as densely forested estuarine areas are being reported to be point source of antibiotic resistance.

Objectives

The objective of this study was to understand the abundance and the dynamics of bacterial antibiotic resistance in the pristine mangrove sediment of Indian Sundarbans, a world heritage site (UNESCO). To achieve this goal we adopted strategies for functional screening of multi-drug resistant bacterial species and made an attempt to elucidate the probable reasons for the spread of this phenomenon in light of *bla*-TEMgene abundance.

Methods

Bacterial strains were isolated, identified and characterized using standard techniques. The effect of antibiotics on multi-drug resistant bacterial isolates was further quantitatively evaluated in both planktonic and biofilm lifestyles. Furthermore, the population dynamics of multi-drug resistant bacteria in the sediment was assessed quantitatively using the q-PCRtechnique.

Conclusions

Results:

117 ampicillin resistant bacteria were isolated including 18 multi-drug resistant strains. Environment typical (*Halobacillus, Jeotgalicoccus*) and atypical (*Bacillus, Staphylococcus*) organisms were found among the isolates showing high MIC to different antibiotics.The q-PCR results showed 0.6%-4.2% abundance of the*bla*-TEM gene in thesediment microbiome. Biofilm dynamics were found to be species and drug specific.

Conclusions:

The results showed a high incidence of multi-drug resistance in the isolated strains. The phylogenetic interrelatedness of the *bla*-TEM genes indicated a possible common primary ancestor. Such a distribution of *bla*-TEM could be achieved through possible horizontal gene transfer. The biofilm lifestyle plays a key role in the development of considerable resistance in the isolated organisms.

FEMS7-3328
One health initiative

FINDING THE BALANCE – CONVERGENCE IN HEALTH DISCIPLINES
K. Queenan[1], R. Kock[1]
[1]Royal Veterinary College, Pathobiology and Population Sciences, Hatfield, United Kingdom

Finding the balance – convergence in health disciplines

Richard A Kock Professor Wildlife Health and Emerging Diseases and Kevin Queenan

Royal Veterinary College London rkock@rvc.ac.uk

Pressures for novel approaches for human, animal and environmental health are arising from a number of directions and from a perception that emerging diseases are threatening reversal in gains made in health generally over the 20th Century. Concerns relate both to the global rise in infectious disease outbreaks in the last decades (Smith et al 2014) and the (re-) emergence of human and animal pathogens and antimicrobial resistance, as well as non-communicable diseases. The latter are commonly associated with urbanisation of populations and changing food systems leading to increasing malnutrition, rising physical and mental health concerns and associated diseases such as obesity, allergies, air pollution-induced lung disease and gastrointestinal disease from a shifting microbiome (Rook 2013). Moreover, there are concerns about ecosystems health, threatened populations of a number of plant and animal species and a degrading environment affecting biodiversity and the ability to produce food in a sustainable way.

All these emergent health problems show complex epidemiologies, involve multiple, closely interrelated species and risk factors. Proponents of One Health state that modern problems require a holistic systemic approach, beyond the current focus on sectoral, technological solutions to individual diseases, and towards a better understanding of the current drivers of ill-health and of the changing environment and interface between species (Fischer et al 2016). It is proposed that a One Health approach is necessary to tackle the challenges through accepting that their complexity requires interdisciplinarity, in particular applying natural and social sciences to human and animal health in the context of a sustainable environment and engaging the whole of society in all aspects. Hence, One Health promotes an integrated approach to health that aims to break up artificial boundaries created by disciplines and sectors.

Underpinning the paradigm is recognition of the importance of resilience in any biological system: factors that erode resilience are critical to loss of equilibrium and to systems change which can be expressed in terms of health at any scale (Horowitz and Wilcox 2005). Sheldon & Verhulst (1996) described mechanisms whereby the partitioning of finite energy resources among competing, costly physiological functions, are a prime cause of variation in immune defences. Possible examples of this are in arctic species responses to a rapidly shifting climate, where extraordinary energy demands driven by climate extremes, lead apparently to a collapse in immune function, raised parasite opportunism and mass mortality events (Kutz et al 2015; Fey et al., 2015).

Studies directed at this interface are now taking off but there are many institutional, disciplinary, financial (Bromham et al 2016) and societal constraints on One Health which will need to be overcome. Academic initiatives are contributing to the development of methods and frameworks for action (Coker at al., 2011; Min B. et al., 2013) including regional alliances or projects such as the Network for Evaluation of One Health (NEOH), the International Association of Ecological Health, the USA based One Health Initiative, the One Health Platform and a variety of other smaller groups. The veterinary profession has been a significant contributor to this process, along with Public Health professionals and it is gradually attracting a wider community of scientists and practitioners with objectives ranging from establishing collaboration between clinicians and researchers in the human and animal field, to deeper examination of the political economies of health in a more structural socio-political context (Wallace et al., 2015). The growing interest in this area is also reflected in the number of new academic courses including at under- and postgraduate level in One Health. There is for example a joint face-to-face joint Masters course at the Royal Veterinary College and London School of Hygiene and Tropical Medicine, now in its fourth year drawing students from veterinary, medical, biomedical, pharmacy, natural sciences, psychology, political science and economics backgrounds.

While the One Health paradigm is still evolving and causes controversy over its definition (Kingsley and Taylor 2016; Mi et al., 2016), there is a general agreement that modern challenges need integrated, systems-based approaches. Conversion of science into policy in the One Health arena will require some radical changes in emphasis in both the academic and practical expression of health sciences and systems.

FEMS7-0126
One health initiative

ONE HEALTH: PLANTS, ANIMALS, HUMANS, AND FOODBORNE DISEASE

S. Maloy[1]
[1], *USA*

Although infectious diseases have always plagued humans, the frequency of emerging and re-emerging infectious disease has been increasing over the last several decades. These diseases are typically caused by (i) new pathogens that are transmitted to humans or animals following environmental disturbance or acquisition of novel virulence traits, or (ii) existing and known pathogens that have spread to new geographic areas or populations. Understanding how and why emerging pathogens arise has relied heavily on genomic and metagenomic approaches. Environmental reservoirs of virulence genes, such as exotoxin genes and antibiotic resistance genes, provide the raw materials for evolution of new pathogens following horizontal gene transfer to new hosts or exposure of naïve hosts to opportunistic pathogens from the environment. Evolution of new strains of *Salmonella enterica* and *Escherichia coli* clearly demonstrate the impact of disruption of the environment on transmission to animals and humans. The One Health initiative integrates insights from environmental science, veterinary medicine, and human medicine to develop upstream approaches to prevent disease. Several examples will demonstrate how implementation of One Health approaches can prevent the transmission of food-borne diseases.

FEMS7-1669
One health initiative

BIOCONTAMINATION OF SURFACES AND WATERS INSIDE THE INTERNATIONAL SPACE STATION

E. Perrin[1], G. Bacci[1], A. Mengoni[1], F. Canganella[2], G. Bianconi[2], L. Garrelly[3], S. Amalfitano[4], C. Levantesi[4], S. Rossetti[4], S. Perero[5], M. Ferraris[5], R. Fani[1]
[1]University of Florence, Department of Biology, Sesto Fiorentino Florence, Italy
[2]University of Tuscia, Department of Biological- Agrofood and Forestry sciences DIBAF, Viterbo, Italy
[3]GL-Biocontrol, GL-Biocontrol, Clapiers, France
[4]National Research Council of Italy, Water Research Institute, Monterotondo Roma, Italy
[5]Polytechnic of Turin, Department of Applied Science and Technology DISAT, Torino, Italy

Backgrounds

Space exploration requires the development of methods for preventing, monitoring and controlling biocontamination within human confined environments. These methods need to be automated, simple, lightweight and with minimal consumables. Space and terrestrial monitoring as well as prevention/mitigation methods are currently working separately, rather than in synergy. The Horizon 2020 Biowyse project foresees development and demonstration of an integrated biocontamination control system for water and humid areas.

Objectives

Previous experiments provided valuable information for the Biowyse project. The Viable ISS (financially sponsored by the Italian Space Agency and supported by NASA) study involves the evaluation of the microbial biofilm development on space materials. Samples included in Viable ISS are composed of both metallic and textile space materials, that are placed both inside and outside of four foam lined Nomex bags, each one subjected to a different pre-treatment procedure. These bags were exposed inside International Space Station from 2011 to 2016. Vials with samples of potable water used on ISS were also included.

Methods

Different methodologies were used for the determination of the bacterial load on surfaces and water samples (cultivation on LB agar, ATP-metry, Flow-Cytometry, qPCR). The composition of the microbial communities was determined through 16S rDNA high-throughput sequencing.

Conclusions

Silver pre-treatment were found to be the most effective, while the microbiota composition was mainly influenced by human presence.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 687447

FEMS7-2354
One health initiative

A FRAMEWORK FOR GUT-BRAIN AXIS ANNOTATION FROM METAGENOMIC DATA

M. Valles-Colomer[1], *J. Raes*[1]
[1]*VIB - KU Leuven, Department of Microbiology and Immunology- Rega Institute, Leuven, Belgium*

Backgrounds

There is evidence of bidirectional communication between the gut microbiota and the nervous system, potentially playing a role in brain development, brain physiology, and behavior. One of the main mechanisms of gut-brain communication is the metabolism of neuroactive compounds by the gut microbiota, with either a beneficial or detrimental effect on the host. Although metagenomic sequencing provides both insights into species composition and their functional potential, dedicated tools are needed to facilitate gut-brain axis systematic analysis and interpretation.

Objectives

To identify the microbial functional properties involved in the gut-brain axis, and assess associations between microbial features and mental wellbeing from human fecal metagenomic datasets.

Methods

We assembled a metabolic reconstruction framework describing the microbial pathways leading to synthesis or degradation of nervous system-interacting compounds based on extensive literature review. This manually-curated framework consists of a set of gut-brain modules (GBM), each containing the alternative enzymes (orthologous groups) associated to the conversion of a certain neuroactive compound by the gut microbiota. After assessing GBM distribution in gut microbial reference genomes, we applied the framework in a general population cohort (N=1100) to identify associations between gut microbial functional potential and wellbeing indicators.

Conclusions

We assembled a gut-brain analysis framework and by applying it in a large general population cohort we identified bacterial functional properties (GBMs) and taxonomic groups that are significantly associated with host wellbeing.

FEMS7-0164
Plant-bacteria interactions under omics spotlights

THE PSEUDOMONAS PUTIDA TYPE VI SECRETION SYSTEM (T6SS) IS A PLANT WARDEN AGAINST PHYTOPATHOGENS

P. Bernal[1], L.P. Allsopp[1], M.A. Llamas[2], A. Filloux[1]
[1]Imperial College London, Life Sciences, London, United Kingdom
[2]Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas, Environmental Protection, Granada, Spain

Backgrounds

Bacterial type VI secretion systems (T6SSs) are molecular weapons designed to deliver toxic effectors into prey cells. These nanomachines play an important role in inter-bacterial competition and provide advantages to T6SS active strains in polymicrobial environments.

Objectives

Here we analyse the genome of the biocontrol agent *Pseudomonas putida* KT2440 and identify three T6SS gene clusters (K1-, K2- and K3-T6SS). Besides, ten T6SS effector/immunity pairs were found, including putative nucleases and pore-forming colicins. We show that the K1-T6SS is a potent antibacterial device which secretes a toxic Rhs-type effector Tke2. Remarkably, *P. putida* eradicates a broad range of bacteria in a K1-T6SS-dependent manner, including resilient phytopathogens which demonstrates that the T6SS is instrumental to empower *P. putida* to fight against competitors. Furthermore, we observed a drastically reduced necrosis on the leaves of *Nicotiana benthamiana* during co-infection with *P. putida* and *Xanthomonas campestris*. Such protection is dependent on the activity of the *P. putida* T6SS.

Methods

We have used a battery of bioinformatic tools including Phyre2 to perform structural-base homology prediction, PyMOL to build structural alignments and MEGA6 to construct the phylogenetic tree. Secretion, growth inhibition and *in vitro* inter-bacterial competition assays were performed. *In planta* bacterial competition assays were carried out on *Nicotiana benthamiana* leaves.

Conclusions

Many routes have been explored to develop biocontrol agents capable of manipulating the microbial composition of the rhizosphere and phyllosphere. Here we unveil a novel mechanism for plant biocontrol which needs to be considered for the selection of plant wardens whose mission is to prevent phytopathogen infections.

FEMS7-3304
Plant-bacteria interactions under omics spotlights

QUORUM-SENSING AND QUORUM-QUENCHING IN PLANT HOST-PATHOGEN INTERACTIONS

D. Faure[1]
[1]*CNRS, I2BC, Gif Sur Yvette, France*

Quorum-sensing (QS) relies cell density and gene expression in a wide range of bacteria which interact with plants, including symbionts and pathogens. Quorum quenching encompasses all the processes that disturb QS signaling, such as QS-signal degrading enzymes and QS inhibition compounds that may be produced by the plant host or microbiota. QS and quorum quenching molecular actors will be presented, and their involvement in host-bacteria interactions will be exemplified, as well as their potential uses for protecting plants.

FEMS7-2503
Plant-bacteria interactions under omics spotlights

THE TRIPARTITE SYMBIOSIS OF PIRIFORMOSPORA INDICA, THE ENDOBACTERIUM RHIZOBIUM RADIOBACTER AND CROP PLANTS - TRANSCRIPTOME STUDIES OF DI- AND TRIPARTITE INTERACTIONS WITH BARLEY

S.P. Glaeser[1], H. Guo[2], S.F. Zanini[2], E. Secic[2], I. Alabid[2], J. Imani[2], M. Hardt[3], T. Busche[4], M. Rothballer[5], J. Blom[6], A. Goesmann[6], J. Kalinowski[4], A. Hartmann[5], P. Kaempfer[1], K.H. Kogel[2]
[1]Justus Liebig University of Giessen, Institute of Applied Microbiology, Giessen, Germany
[2]Justus Liebig University of Giessen, Institute of Phytopathology, Giessen, Germany
[3]Justus Liebig University of Giessen, Biomedical Research Centre Seltersberg-Imaging Unit, Giessen, Germany
[4]Bielefeld University, Microbial Genomics and Biotechnology- CeBiTec, Bielefeld, Germany
[5]Helmholtz Zentrum München- German Research Center for Environmental Health, Research Unit Microbe-Plant Interactions, Neuherberg, Germany
[6]Justus Liebig University of Giessen, Bioinformatics and Systems Biology, Giessen, Germany

Backgrounds

The growth-promoting fungus *Piriformospora indica* harbors an endobacterium which is frequently detected in low abundance in fungal lab cultures by fluorescence in situ hybridization and quantitative PCR. The endobacterium was isolated and identified as *Rhizobium radiobacter* (syn. *Agrobacterium tumefaciens*). While the endobacterium grows in pure culture (strain *Rr*F4), the fungus could not be completely cured. Thus, the role of the endobacterium in the tripartite symbiosis with plants is still unclear.

Objectives

First, genome data were generated and root colonization studies were performed to learn more about the function of the endofungal bacterium. In contrast to other endofungal bacteria, the genome of *Rr*F4 is not reduced in size. Instead, it shows high similarity to the genome of the plant pathogenic *A. tumefaciens* C58, except vibrant differences in tumor-inducing (pTi) and accessory (pAt) plasmids, explaining the loss of pathogenicity. Similar to *P. indica*, *Rr*F4 promotes plant growth and induces systemic resistance in plants. GUS- and GFP-tagged *Rr*F4 showed the proliferation of *Rr*F4 in axenic barley and Arabidopsis roots colonizing the rhizodermis and cortical tissue of the root hair zone similar to *P. indica*, but, unlike its fungal host, *Rr*F4 can penetrate into the root stele typical for plant growth promoting bacteria

Methods

Transcriptome data were generated from axenic culture experiments of tripartite interactions of *P. indica,* the endobacterium and barley and dipartite interactions of *Rr*F4 and barley.

Conclusions

The barley transcriptome gave a first inside into the microbe specific response of barley and the transcriptome of *Rr*F4 showed for the first time the specific biological activity of the endobacterium on crop plants if it is released from the fungal host.

FEMS7-3305
Plant-bacteria interactions under omics spotlights

SMALL RNA DISCOVERY IN PLANT PATHOGENS AND SYMBIONTS

F. Narberhaus[1]
[1]*Ruhr University Bochum, Microbial Biology, Bochum, Germany*

Small regulatory RNAs (sRNAs) control gene expression in all domains of life, and are important regulators in plant-associated bacteria (1). Using next-generation sequencing approaches, we and others identified more than 600 sRNA candidates in the phytopathogen *Agrobacterium tumefaciens* (2,3,4). Several of them are differentially expressed in response to growth or stress conditions suggesting a regulatory function. The sRNA AbcR1 (ABC regulator1) accumulates during stationary phase and controls the expression of multiple ABC transporters by two distinct single-stranded regions (5,6). One of its negatively-controlled targets is the *atu2422* mRNA, which encodes a substrate binding protein for proline and gamma-aminobutyric acid (GABA), a plant-secreted defence molecule. The absence of AbcR1 resulted in accumulation of Atu2422 and increased GABA import (5).

Recent results on AbcR1, another sRNA that also controls ABC transporter genes, and a sRNA that regulates growth, motility and antibiotic resistance will be presented.

1. Becker A, Overlöper A, Schlüter JP, Reinkensmeier J, Giegerich R, Narberhaus F, Evguenieva-Hackenberg E. 2014. Riboregulation in plant-associated alpha-proteobacteria. RNA Biology 11:550-562.

2. Wilms I, Overlöper A, Nowrousian M, Sharma CM, Narberhaus F. 2012. Deep sequencing uncovers numerous small RNAs on all four replicons of the plant pathogen *Agrobacterium tumefaciens*. RNA Biology 9:446-457.

3. Lee K, Huang X, Yang C, Lee D, Ho V, Nobuta K, Fan JB, Wang K. 2013. A genome-wide survey of highly expressed non-coding RNAs and biological validation of selected candidates in *Agrobacterium tumefaciens*. PloS One 8:e70720.

4. Dequivre M, Diel B, Villard C, Sismeiro O, Durot M, Coppée JY, Nesme X, Vial L, Hommais F. 2015. Small RNA deep-sequencing analyses reveal a new regulator of virulence in Agrobacterium fabrum C58. Mol Plant Microbe Interact 28:580-589.

5. Wilms I, Voss B, Hess WR, Leichert LI, Narberhaus F. 2011. Small RNA-mediated control of the *Agrobacterium tumefaciens* GABA binding protein. Mol Microbiol 80:492-506.

6. Overlöper A, Kraus A, Gurski R, Wright PR, Georg J, Hess WR, Narberhaus F. 2014. Two separate modules of the conserved regulatory RNA AbcR1 address multiple target mRNAs in and outside of the translation initiation region. RNA Biology 11:624-640.

FEMS7-0406
Plant-bacteria interactions under omics spotlights

IMPACT OF PLANT DOMESTICATION ON MICROBIOME ASSEMBLY

J.M. Raaijmakers[1], J. Perez[1], V. Carrion[1]
[1]NIOO-KNAW, Microbial Ecology, Wageningen, The Netherlands

Backgrounds

The rhizosphere microbiome is pivotal for plant growth and health, contributing to nutrient acquisition and stress tolerance. Microbiome assembly is driven in part by the plant genotype, but little is known about the impact of plant domestication on microbiome assembly of economically important food crops.

Objectives

Here, we investigated rhizobacterial community composition in modern and wild accessions of common bean (*Phaseolus vulgaris*) grown in agricultural soil from the highlands of Colombia, one of the centers of common bean diversification.

Methods

DArT-based genotyping and phenotyping of local common bean accessions showed substantial genetic and root architectural differences between wild and modern bean accessions. Wild accessions showed a higher specific root length and root density than the landrace and modern accessions. Rhizobacterial community analyses combined with modeling showed that species abundance is explained by niche-based distributions, with 13.5% of the variability in community composition determined by the bean genotype.

Conclusions

Along the bean genotypic trajectory, going from the most ancestral to the most modern (based on inbreeding coefficient and homozygosity), we observed a gradual decrease in the relative abundance of Bacteroidetes, mainly Chitinophagaceae and Cytophagaceae, and an increase in relative abundance of Actinobacteria and Proteobacteria, in particular Nocardioidaceae, and Rhizobiaceae. Collectively, the results indicate that domestication of common bean affected rhizobacterial community assembly. The impact of these microbiome shifts on plant growth and health will be discussed.

FEMS7-2666
Profiling the functional landscape of bacterial cells

CRISPR-CAS SYSTEMS INTERACTIONS WITH DNA REPAIR PATHWAYS SHAPE CRISPR-CAS SYSTEMS DISTRIBUTION AMONG BACTERIAL GENOMES

A. Bernheim[1], C. Basier[2], A. Calvo Villamanan[2], E.P. Rocha[1], M. Touchon[1], D. Bikard[2]
[1]Institut Pasteur, Genomes and Genetics, Paris, France
[2]Institut Pasteur, Microbiology, Paris, France

Backgrounds

CRISPR-
Cas systems confer bacteria and archea an adaptative immunity against phages and other invading g enetic elements playing an important role in bacterial evolution. Only 47% of bacterial genomes harbor a CRISPR-Cas system despite their high rate of horizontal transfer. Hypothesis such as the cost of autoimmunity or the trade off between a constitutive or an inducible defense system have been put forward to explain this paradox.

Objectives

We propose that the genetic background plays an important role in the process of maintaining a CRISPR-Cas system after its transfer. More precisely we hypothesized that CRISPR-Cas systems interact with DNA repair pathways.

Methods

To test this idea, we detected DNA repair pathways and CRISPR-Cas systems in bacterial genomes and studied their co-occurences. We report both positive and negative associations that we interpret as potential antagonistic or synergistic interactions. We then focused on one interaction (Type II-A and NHEJ) to validate our result experimentally and explore molecular mechanisms behind those interactions.

Conclusions

Our results suggest that the presence of NHEJ impairs Type IIA CRISPR-Cas systems by limiting the acquisition of new spacers and that conversely type II-A CRISPR-Cas systems limits NHEJ efficiency. Our findings give insights on the complex interactions between CRISPR-Cas systems and DNA repair mechanisms in bacteria and provide a first example on the necessity of accommodation of CRISPR-Cas systems to a specific genetic context to be selected and maintained in bacterial genomes.

FEMS7-0146
Profiling the functional landscape of bacterial cells

TRANSCRIPTION PROFILE OF THE BACTERIAL GENOME: A REVOLUTIONARY PARADIGM
A. Ishihama[1]
[1]*Hosei University- Micro-Nano Technology Research Center, Frontier Bioscience, Koganei- Tokyo, Japan*

Transcription Profile of the Bacterial Genome: A Revolutionary Paradigm
Akira ISHIHAMA

Hosei University, Micro-Nano Technology Research Center, Tokyo 184-8584, Japan

After the complete genome sequencing, the research frontier of bacterial molecular genetics shifted toward understanding the regulation of whole set of genes on the genome in bacteria growing under stressful environments in nature. The model prokaryote, *Escherichia coli*, contains a total of more than 4,500 genes, but the total number of RNA polymerase (RNAP) is only about 2,000 molecules per genome. The regulatory targets of RNAP are, however, modulated through modulation of its promoter selectivity after two-steps of the protein-protein interplay with 7 species of the sigma factor in the first step, and more than 300 species of the transcription factor (TF) in the second step. To get insight into the molecular mechanisms underlying transcription regulation of the genome, we have been involved for more than 20 years for identification of the regulatory targets for all sigma factors and all TFs using two newly developed *in vitro* systems: 1) the Genomic SELEX screening of the regulatory targets for each regulator, and 2) the PS-TF screening for identification of the regulators controlling each promoter. In parallel, we determined the intracellular concentrations of all these regulatory proteins. Noteworthy is that all these experiments were carried with use of a single and the same *E. coli* K-12 strain. Taken all these data together, we will be able to predict the expression of all genes in the *E. coli* genome under given conditions. Here I will overview the current state of our research of the regulation of genome transcription and some novel findings.

FEMS7-2357
Profiling the functional landscape of bacterial cells

PROFILES OF ANTISENSE TRANSCRIPTION WITHIN GENES CODING FOR REGULATORY PROTEINS IN ESCHERICHIA COLI

N. Markelova[1,2], M. Tutukina[1,3], U. Shvyreva[1], I. Masulis[1], F. Kondrashov[3], O. Ozoline[1]
[1]Institute of Cell Biophysics RAS, Functional genomics and cellular stress, Pushchino, Russia
[2]Pushchino State Institute of Natural Sciences, Cell biology, Pushchino, Russia
[3]Centre for Genomic Regulation CRG and Universitat Pompeu Fabra UPF, Bioinformatics and genomics programme, Barcelona, Spain

Backgrounds

Evolution of whole-genome techniques allowed to discover thousands of potential non-coding RNAs, including those transcribed in antisense direction. In E. coli, their ratio to the total amount of annotated genes can reach 22%. The reasons of such a widespread antisense transcription and possible mechanisms for asRNAs' action are not yet understood.

Objectives

The aim of this work was to analyse the profile of asRNAs within the genes coding for transcription factors in E. coli strains growing on different carbon sources.

Methods

E. coli K-12 MG1655 wt, exuR and dps mutants were grown aerobically on M9+0.2% glucose/glucuronate for 4/8 hours. RNA was isolated using Ambion RNAqeous kit. cDNA libraries were prepared and then sequenced on Illumina HiSeq2000 as 50nt single end reads. Data were analysed using Matcher GeneCount.

Conclusions

We analysed profiles of 160 E. coli MG1655 genes coding for transcription factors (76% from all annotated regulators). Antisense transcription was registered within most of the studied loci and was absent only in 18 genes (11%). In most cases, both constant asRNAs and those responding to environmental changes, such as carbon source and growth phase, were registered. Effects of two regulatory proteins were also detected: ExuR as a metabolic regulator, and Dps as a structural protein. The most striking effect was registered for leuO, which transcription in both sense and antisense direction was significantly downregulated by Dps. Being in line with a concept of widespread antisense transcription, our findings do not fully support the idea that bacterial asRNAs are the products of transcriptional noise.

FEMS7-3286
Profiling the functional landscape of bacterial cells

DISCOVERING NOVEL GENES AND FUNCTIONS IN BACTERIA BY COMBINED RNASEQ AND RIBOSEQ

K. Neuhaus[1], *S. Scherer*[2]
[1]*ZIEL Institute for Food & Health, Core Facility Microbiome/NGS, Freising, Germany*
[2]*Technische Universität München, Lehrstuhl für Mikrobielle Ökologie, Freising, Germany*

While NGS allows rapid global detection of transcripts, it remains difficult to distinguish ncRNAs from short mRNAs. To detect potentially translated (novel) RNAs in enterohemorrhagic *E. coli* (EHEC), we used an improved protocol for bacterial ribosomal footprinting (RIBOseq). This allowed distinguishing ncRNA from mRNA. A high ratio of ribosomal footprints per transcript (ribosomal coverage value, RCV) is expected to indicate a translated RNA, while a low RCV should point to a non-translated RNA.

Based on their low RCV, novel non-translated EHEC transcripts were identified as putative ncRNAs, representing both antisense and intergenic transcripts. A number of those novel ncRNAs had expressed homologs in *E. coli* MG1655. Surprisingly, the annotated ncRNAs in EHEC, several showed an RCV similar to protein-coding genes, had RIBOseq patterns matching annotated genes in other enterobacteriaceae, and seem to possess a Shine-Dalgarno sequence. Taken together, this suggests that such ncRNAs may encode small proteins instead of being solely non-coding.

Finally, we found a larger number of intergenic novel ORFs that are translated. These novel ORFs are generally shorter and, therefore, might have been missed by previous annotations and experiments. Thus, RIBOseq is a powerful tool to detect and analyze translational events.

FEMS7-2733
Profiling the functional landscape of bacterial cells

THE ROLE OF THE ESCHERICHIA COLI GENERAL STRESS RESPONSE IN ADAPTING TO LONG TERM RESIDENCE IN A SOIL ENVIRONMENT

C. O'Byrne[1], *Y. Somorin*[1]
[1]*Bacterial Stress Response Group, Microbiology, Galway, Ireland*

Backgrounds

Studies have shown that RpoS, the regulator of the general stress response in *Escherichia coli*, can lose functionality through mutation of the *rpoS* gene when nutrients are limiting or during routine laboratory culture. A unique collection of long-term soil-persistent *E. coli* strains was investigated to determine if their general stress response had been altered during the adaptation to a comparatively nutrient poor soil environment.

Objectives

The study investigated the role of RpoS in soil adaptation and also the role of curli-mediated biofilm.

Methods

The study investigated the integrity of the *rpoS* locus as well as the expression and activity of RpoS. Mutants lacking *rpoS* were constructed and their ability to survive in soil was assessed. The role of RpoS in surviving predation by protozoa was also determined. Biofilm and curli expression was measured in soil adapted strains and correlated with their ability to attach to soil particles.

Conclusions

RpoS was found to be fully conserved in the soil strains and mutants lacking this sigma factor were highly compromised for soil survival. RpoS was found to play a role is resisting low moisture content and in protecting against protozoal predation. Some strains were found to produce limited biofilm and these were shown to be defective for curli expression. Whole genome sequence analysis of these strains revealed alterations in c-di-GMP signalling in these strains. The results suggest that loss of curli expression could contribute to dissemination in a soil environment.

FEMS7-0540
Profiling the functional landscape of bacterial cells

DISSECTING NUCLEATION, GROWTH AND INHIBITION OF BACTERIAL FUNCTIONAL AMYLOIDS AT SINGLE FIBER RESOLUTION

I. Van den Broeck[1], M. Sleutel[1], N. Van Gerven[1], H. Remaut[1], C. Feuillie[2], Y. Dufrêne[2], C. Valotteau[2]
[1]Vrije Universiteit Brussel, Structural Biology Brussels, Brussels, Belgium
[2]Université catholique de Louvain, Institute of Life Sciences, Louvain-la-Neuve, Belgium

Backgrounds

Curli are functional amyloids produced by proteobacteria as part of the extracellular polymer matrix that holds cells together into biofilms.

Objectives

The molecular events during curli nucleation and fiber extension remain largely unknown.

Methods

Here, we combine observations from curli amyloidogenesis in bulk solutions with real-time *in situ* nanoscopic imaging at the single fiber level.

Conclusions

We show that curli display polar growth, and we detect two kinetic regimes of fiber elongation. At high concentration, fibers exhibit steady-state kinetics with a constant instantaneous rate of growth. At low subunit concentration, however, single fibers exhibit stop-and-go dynamics characterized by bursts of steady growth, alternated with periods of stagnation. Curli follow a one-step nucleation process, where monomeric species contemporaneously fold and oligomerize into minimal fiber units that have growth characteristics identical to the mature fibrils. Kinetic data and interaction studies of curli fibrillation in the presence of the natural inhibitor CsgC show the inhibitor binds curli fibers and predominantly acts at the level of fiber elongation.

FEMS7-2080
Surprises in microbial metabolism

ETHANOLAMINE UTILIZATION IN STREPTOMYCES COELICOLOR M145.

A. Bera[1], S. Krysenko[1], A. Matthews[1], N. Okoniewski[2], A. Kulig[1], W. Wohlleben[1]
[1]Interfaculty Institute of Microbiology and Infection Medicine, Microbiology and Biotechnology, Tübingen, Germany
[2]Interfaculty Institute of Microbiology and Infection Medicine, Microbial Bioactive Compounds, Tübingen, Germany

Backgrounds

Non-motile, soil-dwelling *Streptomyces* are able to withstand unfavorable, rapid changing conditions despite constant confrontation with nutrient limitation and pollutants in the soil. Primary nitrogen compounds assimilated by actinobacteria include: ammonium, nitrate, amino acids, urea, amino sugars and peptides. Our studies revealed that *S. coelicolor* can also utilize ethanolamine as a sole nitrogen source. Ethanolamine is a major constituent of bacterial and eukaryotic cell membranes in the form of phosphatidylethanolamine. The well-established model for bacterial ethanolamine utilization involves ethanolamine ammonia-lyase. However, *S. coelicolor* lacks this enzyme and uses so far unknown pathway for the ethanolamine utilization.

Objectives

Here, we report discovery and characterization of a novel gene *glnA4* encoding a glutamine synthetase like enzyme (γ-glutamyl-ethanolamide synthetase) involved in the first step of the ethanolamine utilization pathway in *S. coelicolor.* The GlnA4 catalyzes an ATP-dependent condensation of ethanolamine with L-glutamate to form γ-glutamyl-ethanolamide. We could show that this enzyme accepts as a substrate also ethylendiamine and ethylamine, resulting in the synthesis of the γ-glutamyl-ethylamineamide and γ-glutamyl-ethylamide (L-theanine), respectively.

Methods

The GlnA4 has been overexpressed, purified and characterized biochemically using an *in vitro* assay based on GS activity assay. The product of the reaction catalyzed by GlnA4 was confirmed by HPLC/MS. The deletion of *glnA4* resulted in a growth defect on ethanolamine as a sole nitrogen source. The *glnA4* mutant accumulated ethanolamine intracellularly as confirmed by HPLC. RT-PCR revealed that the expression of *glnA4* was induced by ethanolamine and repressed by ammonium.

Conclusions

First step of the novel ethanolamine utilization pathway was characterized in *S. coelicolor*.

FEMS7-1285
Surprises in microbial metabolism

DECIPHERING A BACTERIAL METABOLIC PATHWAY: THE AEROBIC TRIGONELLINE DEGRADATION PATHWAY IN A. BAYLYI ADP1

N. Perchat[1], P.L. Saaidi[1], E. Darii[1], C. Pelle[1], M. Besnard-gonnet[1], V. De Berardinis[1], M. Dupont[1], A. Gimbernat[1], M. Salanoubat[1], A. Perret[1], C. Fischer[1]
[1]CEA-IG UMR8030 CEA/UEVE/CNRS, UMR8030 CEA/UEVE/CNRS, Evry Cedex, France

Backgrounds

Unknown metabolic pathways are still hampering functional annotation of genomes and experimental research is needed for providing access to gene function.

Objectives

We aimed to investigate the undescribed metabolism of trigonelline (N-methylnicotinate), a pyridine compound synthesized by plants to resist draught stress that soil bacteria use as a carbon, nitrogen and energy source. Our laboratory focuses on the experimental search of gene function by addressing these questions in *A. baylyi* ADP1. This gram-negative, non-pathogenic and nutritionally versatile strictly aerobic soil bacterium is easily amenable to genetic manipulations due to its natural competency.

Methods

By using a complete single-gene deletion mutant collection, previously constructed in our laboratory, we identified a cluster of genes involved in the degradation of trigonelline. The role of each enzyme of the pathway was then investigated through an untargeted LC/MS-based metabolomic approach and further refined by solving the structure of the different catabolic intermediates (some of them being new metabolites, not present in the databases) and performing enzymology studies with the recombinant proteins.

Conclusions

The breakdown of the N-methylpyridine ring provides succinate and methylamine, which are further catabolized by the cells. The wide occurrence of this pathway in bacteria of soil and marine origins emphasizes the ecological importance of this overlooked compound. Our work is contributing to the functional annotation of genomes and expands our knowledge of the bacterial metabolite repertoire.

FEMS7-1041
Surprises in microbial metabolism

GLUTATHIONE TRANSFERASES IN DEGRADATION OF LIGNOCELLULOSIC MATERIALS

E. Gelhaye[1], *M. Rouhier*[1]
[1]*Université de Lorraine, UMR 1136 INRA/UL Interactions Arbres / Microorganismes, Vandoeuvre, France*

Backgrounds

Wood decaying fungi have developed unique features allowing them to use complex carbon sources. The adaptation of these organisms to their life style is due at least in part of the existence of extracellular degrading systems. Besides these extensively studied extracellular systems of degradation, wood decaying (and more globally complex organic matter degrading) fungi possess also extended detoxification systems in comparison with other fungi belonging to other trophic types. A comparative genomic approach revealed indeed the presence of an extended multigenic family encoding glutathione transferases (GSTs) in the genomes of wood decayers

Objectives

From structural, biochemical and physiological studies, our aim is to understand the physiological functions of this multigenic family of GSTs in relation with the degradation of complex biomass.

Methods

Phylogenetic analysis.Transcriptomic and proteomic approaches. Biochemical and structural characterization of recombinant proteins. Studies of proteins/ligands interactions.

Conclusions

During the last few years, our group has strongly invested in the biochemical and structural characterization of various isoforms from different fungal models and in particular from *Phanerochaete chrysosporium*. These studies have revealed that the activity of these enzymes could be split off into two functional groups catalyzing opposite reactions, namely: glutathionylation and deglutathionylation. The characterized isoforms are also able to interact with various molecules present in wood from various tree species, such as flavonoids, terpenes] or resulting of the wood biodegradation. Some of these fungal GSTs are also structurally related to the bacterial GSTs involved in lignin degradation. We will discuss the potential use of these GSTs in biotechnology

FEMS7-0454
Surprises in microbial metabolism

FUNCTIONAL CHARACTERIZATION OF 3-KETOSTEROID 9A-HYDROXYLASES IN RHODOCOCCUS RUBER STRAIN CHOL-4

G. Guevara[1], M. Rocha[1], A. Blanco-Rivero[1], J.M. Navarro-Llorens[1], J. Perera[1]
[1]Unversidad Complutense de Madrid, Biochemistry and Molecular Biology I, Madrid, Spain

Backgrounds

The 3-Ketosteroid 9α-Hydroxylase, also known as Ksh [androsta-1,4-diene-3,17-dione, NADH:oxygen oxidoreductase (9α-hydroxylating); EC 1.14.13.142)], is a two component Rieske non-heme monooxygenase made up of the oxygenase subunit (KshA) and the reductase subunits (KshB). KshAB is a key activity in bacterial steroid catabolism in combination with a 3-ketosteroid-Δ^1-deydrogenase activity (KstD), being both responsible of the steroid nucleus (rings A/B) breakage. Ksh initiates the opening of the steroid ring by the 9α-hydroxylation of the C9 carbon of 4-ene-3-oxosteroids (e.g. AD) or 1,4-diene-3-oxosteroids (e.g. ADD), transforming them into 9α-hydroxy-4-androsten-3,17-dione (e.g. 9OHAD) or 9α-hydroxy-1,4-androstadiene-3,17-dione (e.g. 9OHADD), respectively. *Rhodococcus ruber* strain Chol-4 isolated from a sewage sludge sample is able to grow in minimal medium supplemented with steroids, showing a large catabolic capacity. Three different *kshA* and one *kshB* genes have been found in *R. ruber* Chol-4 genome.

Objectives

The aim of this work is the functional characterization of the *ksh* genes found in the Chol-4 strain genome.

Methods

For this work, several methods have been followed: growth studies of single, double and triple mutants on different substrates, mutant complementation and biotransformation studies on resting-cells and grown cells.

Conclusions

KshA2 is needed for the degradation of steroid substrates with short side chain while KshA3 is needed for the degradation of those substrates with longer side chains. KshA1 is a more versatile enzyme related to the cholic acid catabolism but it also collaborates with KshA2 or KshA3 in the general degradation of steroids.

FEMS7-0119
Surprises in microbial metabolism

UTILIZATION OF THE PLANT SUGAR SULFOQUINOVOSE BY BACTERIA PROCEEDS IN METABOLIC ANALOGY TO THE GLUCOSE PATHWAYS

D. Schleheck[1]
[1]*University of Konstanz, Biology Department, Konstanz, Germany*

Sulfoquinovose (SQ; 6-deoxy-6-sulfoglucose) is the polar headgroup of the sulfolipid (SQDG) in all plants, algae and in most photosynthetic bacteria, and SQ degradation by bacteria represents an important component of the biogeochemical sulfur cycling. Two pathways for primary degradation of SQ are known thus far. First, a 'sulfo-glycolytic pathway' as found in *Escherichia coli* K-12, which proceeds in direct analogy to the glycolysis (Embden-Meyerhof-Parnas) pathway; SQ is catabolized *via* 6-deoxy-6-sulfofructose-1-phosphate to dihydroxyacetone phosphate (DHAP), which is utilized for growth, and to 2,3-dihydroxypropane-1-sulfonate (DHPS), which is excreted. Second, an Entner-Doudoroff-type of pathway in *Pseudomonas putida*, in which SQ is catabolized *via* 2-keto-3,6-dideoxy-6-sulfogluconate (KDSG) to pyruvate, which is utilized for growth, and to 3-sulfolactate (SL), which is also excreted. The excreted DHPS and SL can be degraded completely by other aerobic bacteria, i.e., inclusive release of the DHPS/SL-sulfur in form of sulfate. Currently we are revealing, most likely, a third type of pathway for SQ in *Bacillus* species. Further, we explore complete SQ degradation in bacterial communities under anoxic conditions, which yields hydrogen sulfide (H_2S) in contrast to sulfate as with aerobic communities. Hence, it appears that primary SQ degradation proceeds in metabolic analogy to glucose utilization, but excludes a desulfonation reaction, and that anaerobic, fermentative degradation of dietary SQ may be a relevant, but yet unrecognized pathway and source of H_2S in the gut-microbiomes of herbivores and omnivores.

FEMS7-2323
Surprises in microbial metabolism

SMOKE SIGNALS, OR HOW SALMONELLA TURNS OFF ENTEROCOCCUS ANTIBIOTIC RESISTANCE

D. Thomas-Lopez[1], L. Carrilero[1], N. Montero[1], A. Hoefer[1], J. Zivanovic[2], J. Miljkovic[2], M. Filipovic[2], B. Gonzalez-Zorn[1]
[1]Faculty of Veterinary Medicine and VISAVET - Complutense University of Madrid, Animal Health, Madrid, Spain
[2]Institute of Biochemistry and Cellular Genetics - University of Bordeaux, CNRS UMR 5095, Bordeaux, France

Backgrounds

Hydrogen sulfide (H_2S) is a gas produced by many bacteria through the 3MST pathway. Its role in different fields, including antibiotic resistance, is nowadays being elucidated. Interestingly, the pathogen *Salmonella* Typhimurium possesses a second mechanism, classically known as the *phsABC* operon, for the production of H_2S.

Objectives

Our objectives are both to unravel the functions of the *phsABC* operon and to understand the purpose of multiple H_2S producing mechanisms coexisting in *S.* Typhimurium, with regards to the H_2S production, internal sulfhydration, antibiotic resistance and interaction with other species.

Methods

The *phsABC* operon of *S.* Typhimurium ATCC14028 was deleted in-frame by using pKD46, pKD13 and pCP20. *E. faecalis* JH2-2 and *E. faecium* ATCC19434 antibiotic susceptibility tests were performed following official guidelines. When needed, they were incubated in presence of H_2S coming either from *S.* Typhimurium or from a chemical source.

Conclusions

The *phsABC* pathway is the only mechanism used by *S.* Typhimurium to produce extracellular H_2S but it plays a small role in internal sulfhydration. However, when exposing other bacteria to *S.* Typhimurium wild-type and ***Δphs*** strains, we have obtained a surprising result: both *E. faecalis* and *E. faecium*, major nosocomial pathogens worldwide, lose their intrinsic resistance to cephalosporins in the presence of H_2S produced by a physically separated *S.* Typhimurium. Results were confirmed by exposing enterococci to a chemical H_2S source. This astonishing result not only opens the door to new therapeutic options for infections caused by these multiresistant pathogens, but also raises new questions about aerial interaction among microorganisms.

FEMS7-0062
The viral world revisited: from viroids to giant viruses

LESSONS LEARNED FROM GIANT VIRUSES STUDIES

C. Abergel[1], F. Elisabeth[1], S. Jeudy[1], S. Santini[1], M. Legendre[1], J.M. Claverie[1], M. Trauchessec[2], Y. Couté[2]
[1]CNRS-AMU, Structural and Genomic information, Marseille, France
[2]CEA-INSERM-UGA, BIG-BGE, Grenoble, France

Acanthamoeba are infected by a remarkable diversity of large dsDNA viruses the infectious cycles of which have been characterized using genomics, transcriptomics and electron microscopy. Given their gene content and the persistence of the host nucleus throughout their infectious cycle, the Marseilleviridae were initially assumed to fully replicate in the cytoplasm. Unexpectedly, we found that their virions did not incorporate the virus-encoded transcription machinery, making their replication nucleus-dependent. I will present the characterization of the Noumeavirus and Melbournevirus infectious cycle which revealed an unanticipated evolutionary intermediate between exclusively cytoplasmic viruses and nuclear viruses.

FEMS7-0953
The viral world revisited: from viroids to giant viruses

VIROIDS: FILLING THE LOWER SIZE NICHE OF THE BIOLOGICAL SCALE

R. Flores[1]
[1], *Spain*

Discovery around 1970 of potato spindle tuber viroid (PSTVd), the first subviral pathogen with autonomous replication, overthrew viruses from the lowest step of the biological scale. Sequencing of PSTVd revealed its minimal size (359 nucleotides), circular structure with a predicted rod-shaped conformation, and lack of protein-coding ability. This compact conformation, composed by double-stranded segments flanked by loops stabilized by non-canocal interactions, exists *in vivo* and is crucial in replication and trafficking. Most of the approximately 30 viroids known, which resemble PSTVd in having a rod-like structure with a central conserved region (CCR) and nuclear replication, form the family *Posiviroidae*. Avocado sunblotch viroid and three other viroids, which lack a CCR but can form hammerhead ribozymes and replicate in plastids, are grouped into the family *Avsunviroidae.* Viroid replication occurs through a rolling-circle mechanism catalized by host enzymes in the *Pospiviroidae* complemented by ribozymes in the *Avsunviroidae.* The presence in plants infected by members of both families of viroid-derived small RNAs (vd-sRNAs) resembling those generated by Dicer-like enzymes indicate that viroids, like viruses, are targeted by the RNA silencing machinery of their hosts. These vd-sRNAs load the Argonaute core of the RNA-induced silencing complex (RISC), and some containing the pathogenicity determinant of certain viroids direct RISC to cleave specific host mRNAs, providing a plausible mechanism of pathogenesis. Their unique properties make viroids candidates for being molecular relics of the RNA world that preceded the present one based on DNA and proteins. Therefore, viroids are structurally, functionally and evolutionarily independent of viruses.

FEMS7-2492
The viral world revisited: from viroids to giant viruses

SINGLE VIRUS GENOMICS REVEALS THE ´SECRET´VIRUSES THAT MICRODIVERSITY HIDES

F. Martínez-Hernández[1], O. Fornas[2], M. Lluesma Gomez[1], M.J. de la Cruz Peña[1], B. Bolduc[3], J. Martínez Martínez[4], J. Antón[1], J.M. Gasol[5], R. Rosselli[6], F. Rodriguez-Valera[6], M.B. Sullivan[3], S.G. Acinas[5], M. Martínez-García[1]
[1]Univeristy of Alicante, Department of Physiology- Genetics- and Microbiology, Alicante, Spain
[2]The Barcelona Institute for Sciences and Technology,
Flow Cytometry Unit: Pompeu Fabra University UPF and Centre for Genomic Regulation CRG, Barcelona, Spain
[3]The Ohio State University,
Departments of Microbiology and Civil- Environmental and Geodetic Engineering, Columbus, USA
[4]Bigelow Laboratory for Ocean Sciences, Bigelow Laboratory for Ocean Sciences, Maine, USA
[5]Institut de Ciènces del Mar ICM-CSIC-, Institut de Ciènces del Mar ICM-CSIC, Barcelona, Spain
[6]University Miguel Hernández, Departamento de Producción Vegetal y Microbiología-, San Juan, Spain

Backgrounds

Viruses have a central influence on global biogeochemical cycles due to their control of microbial populations. However, many of the uncultured viruses are still unknown, hindering our ability to answer fundamental biological, ecological and evolutionary questions about microbial communities in nature.

Objectives

Here, we adapted single-cell genomic technologies to unveil the genomics of natural viral communities that metagenomics and culturing techniques are unable to reveal.

Methods

More than 2,000 single-viruses were separated, one at a time, using fluorescence-activated virus sorting (FAVS) from natural marine environments (surface and mesopelagic Mediterranean Sea and bathypelagic Atlantic Ocean), and 44 viral Single Amplified Genomes (vSAGs) were randomly selected.

Conclusions

Metagenomics and protein-sharing network analyzes showed that these vSAGs are novel highly abundant and microdiverse viral populations from global ocean virome. This indicates that marine vSAGs best represent the uncultured, predominant viral species and genera that dominate the oceans, overlooked by current methodologies.To analyzed why the representative vSAGs have not discovered before, we simulated different levels of viral microdiversity in a natural population. Results showed that viral assembly of microdiverse populations is complicated and was unable to decipher properly that natural genetic microdiversity. Therefore, our analyzes show that single-virus genomics overcome the limitations of current methodologies, and aid to unravel the ecological roles of those 'yet-hidden and secret' viruses from natural viral communities.

FEMS7-0379
The viral world revisited: from viroids to giant viruses

BACTERIOPHAGES IN HYDRA AND THEIR ROLE IN THE METAORGANISM MAINTENANCE

J. Lange[1], T. Bosch[1], T. Lachnit[1]
[1]Zoological Institute- CAU Kiel, Biology, Kiel, Germany

Backgrounds

All multicellular organisms are associated with a host-specific bacterial community. This association is beneficial for both partners and together they form a complex organism termed “metaorganism”. Previous studies have focused on bacteria as key components, while viruses have been mostly disregarded in this context.

Objectives

We expect phages to play a major role as regulators within metaorganisms. We hypothesize that phages are important drivers of the metaorganism homeostasis and function as a part of the immune system.

Methods

The fresh water polyp *Hydra* is a perfect organism to test this hypothesis because it is associated with both a specific bacterial and viral community. We screened our bacteria culture collection for prophage signatures and show that 50% of *Hydra* associated bacteria contain prophages. Six of them are active and inducible by antibiotics and environmental stressors. With cross-infection experiments we analyzed the host range of these phages, of which three feature a broad host range, including the phage of *Hydras* main colonizer *Curvibacter* sp.. To evaluate the role of this dominant phage in homeostasis we conducted recolonization experiments with germfree *Hydra*. We show that *Curvibacter* phage is also active *in vivo* and have the ability to control bacteria directly on *Hydra*. Furthermore we could show *in vivo* that the phage is not only inducible by environmental stressors but also by foreign bacteria, which is triggered by quorum sensing molecules.

Conclusions

Finally we conclude that phages are important for the maintenance of the metaorganism homeostasis and may also function as a part of the immune system.

FEMS7-2814
The viral world revisited: from viroids to giant viruses

THE FIRST PHLEBO-LIKE VIRUS INFECTING PLANTS: A CASE STUDY ON THE ADAPTATION OF NEGATIVE-STRANDED RNA VIRUSES TO NEW HOSTS

B. Navarro[1], M. Minutolo[2], D. Alioto[2], F. Di Serio[1]
[1]Institute for Sustainable Plant Protection CNR, National Research Council of Italy, Bari, Italy
[2]DIpartimento di Agraria, Università degli Studi di Napoli "Federico II", Portici, Italy

Backgrounds

Concave gum (CG) is a virus-like disease of citrus first described in the early 1930s, the etiology of which is still unknown.

Objectives

Identification of viruses associated with CG disease

Methods

Next generation sequencing of cDNA libraries from symptomatic and non-symptomatic citrus plants, field surveys and phylogenetic analyses

Conclusions

A novel negative-stranded RNA virus has been identified and shown to be closely associated with CG disease. The new virus, tentatively named citrus concave gum-associated virus (CCGaV), has a bipartite genome composed of a negative-sense RNA1 coding for the RNA-dependent RNA polymerase (RdRp), and an ambisense RNA2 encoding the nucleocapside (N) and a putative movement protein (MP). RNA signatures in viral RNAs and phylogenetic analyses of RdRP and N proteins highlighted the close relationships of CCGaV with members of the genus *Phlebovirus,* which are arthropod-transmitted viruses infecting mammals, and with some phlebo-like viruses exclusively infecting arthropods. The putative CCGaV MP has the typical features of MPs grouped in the 30K superfamily and is phylogenetically related to the MPs of the plant-infecting viruses of the family *Ophioviridae*. Based on these data, CCGaV can be considered the first phlebo-like virus infecting plants. Implications of these findings in the evolutionary events that allowed the adaptation of the ancestor negative-stranded RNA virus(es), likely infecting arthropods, to plant hosts will be examined. The need of creating a new genus for classifying this bipartite negative-stranded RNA virus, and the impact of specific detection methods developed in the frame of this study on sanitation and certification programs of citrus will be also discussed.

FEMS7-0398
The viral world revisited: from viroids to giant viruses

THE ROD-SHAPED VIRUS SIRV2 - A MODEL FOR STUDIES ON VIRUS-HOST INTERACTIONS IN HYPERTHERMOPHILIC ARCHAEA

D. Prangishvili[1]
[1]*Institut Pasteur, Department of Microbiology, Paris, France*

Backgrounds

Viruses that parasitize hyperthermophilic archaea, with their astoundingly diverse, unique morphotypes and the vast majority of genes without identified functions and homologues in the extant databases, constitute one of the least understood parts of the virosphere and only recently are starting to gradually reveal their secrets (for review, see Prangishvili, 2013. *Annu. Rev. Microbiol.* 67: 565-585).

Objectives

I will summarize the current knowledge on one of the model systems for studies on hyperthermophilic archaeal viruses, the *Sulfolobus islandicus* rod-shaped virus 2, SIRV2 —a member of the family *Rudiviridae*—, and discuss the recent advances towards understanding molecular details of its life cycle, including virion entry, morphogenesis and egress, as well as viral genome transcription and replication.

Methods

Diverse

Conclusions

The morphological and genomic distinctiveness of hyperthermophilic archaeal viruses extends to the mechanisms underlying their interactions with the host cells.